Research Article

Study of serum leptin levels in patients with type 2 diabetes mellitus at a tertiary care centre of central India

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Abstract

Background: It has been reported that Insulin and leptin share a common central signaling pathway and there might be a possible role of leptin in the etiology of type 2 DM. Studies regarding role of leptin in type 2 DM in central India are lacking. **Aim:** This study was conducted with the aim of comparing leptin levels in patients with type 2 DM with apparently healthy controls. **Subjects and Methods:** A total of 80 subjects including 40 patients of type 2 DM attending outdoor clinics of MY Hospital and 40 age and sex matched healthy controls. Fasting venous blood sample was analysed for leptin, total cholesterol (TC), triglycerides (TG), HDL-C and fasting blood sugar (FBS). Statistical analysis was performed using SPSS software version 14. **Results:** Mean BMI of diabetic group was 32 ± 3.48kg/m^2 and that of control group was 28 ± 2.56kg/m^2. Leptin levels among diabetic cases were 29.3± 19.3 ng/ml and in non diabetic subjects it was 34.8 ±21.4 ng/ml. The difference in leptin levels between cases and control were statistically significant. **Conclusion:** Low leptin levels are associated with type 2 diabetes mellitus independent of changes in BMI.

Keywords: Type 2 DM, Leptin, BMI.

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Introduction

Type 2 diabetes mellitus (T2DM), commonly known as an obesity related metabolic disorder, is rapidly emerging as a global health care problem that threatens to reach pandemic levels in a short span of time. India, considered to be the Diabetic capital of the World, the situation is all the more critical. Leptin, a protein hormone expressed and released by adipocytes, is considered to have a role in the regulation of body weight and associated energy metabolism [1].

South Asians including Indians have a high tendency to develop type 2 diabetes even at low BMI. The risk of type 2 diabetes in South Asians is about 4–5 times higher than Europeans. There are defects in insulin secretion and action, and fat metabolism in type 2 diabetes mellitus [2]. Obesity is a well known risk factor for the development of diabetes mellitus. Among the various factors implicated in the etiology of this disease, the role of leptin- the obesity gene product, is increasingly being recognized. Obesity, a state of hyperleptinemia, confers a minimum three to tenfold higher risk of T2DM [2]. These findings draw attention to the possible role of leptin in the etiology of T2DM. It has been reported that Insulin and leptin share a common central signaling pathway [3].

Leptin, a 167 amino acid adipocyte derived hormone, has been implicated in the regulation of adipose mass and has been reported to alter both insulin sensitivity and insulin secretion. Although it is clear that circulating leptin is positively correlated with body fat mass, the relationship of diabetes to plasma leptin concentration, independent of adiposity, is less clear [4,5]. There is controversy about the level of circulating leptin whether it is reduced, raised or remains

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unchanged in type 2 diabetes [6,7]. The variable results in type 2 diabetes are expected because subjects dif fered with respect to extent of obesity, age, gender and ethnic group.

Most of the studies on serum leptin in type 2 diabetes have focused on white populations. In this study we determined leptin concentrations and parameters related to type 2 diabetes mellitus in Indians with type 2 diabetes and normal control subjects.

Subjects and Methods

For the present cross-sectional study, forty patients with type 2 diabetes mellitus and 40 normal control subjects were included in the study. As per the prevalence formula for sample size calculation (n= $4pq/h^2$) minimum sample size required for the study was 27 cases, therefore we have chosen 40 cases for the study, we had limited budget and leptin kit was costly therefore we limited our study to only 80 subjects in total. The patients and controls were matched by ethnic group. Patients had been diagnosed to be diabetic for a median of 5 years (range, 0.5 –20.0 years). They were taken from the outdoor clinics of Maharaja Yashwant Rao Hospital, Indore (M.P.). Type 2 diabetes was defined based on history of patients taking oral hypoglycemic drugs or according to the classification of American Diabetes Association [8] as showing fasting plasma glucose concentration more than 126mg/dl. Controls were apparently healthy volunteers who in the last 12 months were not taking any medication and had no family history of diabetes mellitus. Non-diabetic subjects with endocrine disease, significant renal or hepatic diseases, and those receiving medications that control glucose metabolism, hypertension or hyperlipidemia were excluded from the study. The study protocol was approved by the institutional ethical committee and an informed consent was taken by all the subjects. Anthropometric measurements were assessed in all subjects.

Anthropometric measurements:-

Anthropometric indices including height and weight were taken while subjects were in the standing position and wearing light clothing without shoes. Body weight and height were measured in kilograms and in centimeters, respectively. Body mass index (BMI) was calculated as weight (kilo- grams) divided by height squared (in square meters).

Collection and preparation of sample: With full aseptic precautions 5ml of fasting venous blood sample was collected and allowed it to clot. Clotted blood was centrifuged and clear serum was collected. Fresh serum samples were taken. Serum was checked for hemolysis and if hemolyzed then that serum was discarded. Serum was analysed for leptin, total cholesterol (TC), triglycerides(TG), HDL-C and fasting blood sugar (FBS).

Analytical methods: Lipid profile was assessed by automated analyzer, TC was determined by enzymatic (CHOD-PAP) colorimetric method [9] and TG by enzymatic (GPO-PAP) method [10]. HDL-C was estimated by precipitation method [11], FBS by enzymatic (GOD-POD) and LDL-C by Friedewald formula [12]. Leptin was estimated by sandwich ELISA, using Leptin ELISA kit.

Statistical Analysis: Data was maintained on excel spread sheet. Analysis was performed using SPSS version 14 for windows. Descriptive data were expressed as mean, standard deviation, and range of all variables. Results were presented as mean± S.D. Means of data in patients and controls were compared using the independent t-test. Differences were considered statistically significant at $p<0.05$. Correlation between serum leptin and BMI was sought using the Pearson's correlation.

Results

Eighty subjects, 40 diabetics and 40 non-diabetic controls, were included in this study. Their basic characteristics are given in Table -1. There were 19 women and 21 men as cases and 20 male & 20 females in control group. In diabetic group most of the subjects had hyperglycemia (mean fasting blood glucose 148±52.6 mg/dl), while in non-diabetics it was within normal limits (mean 92±12.4 mg/dl). Mean BMI of diabetic group was 32 ± 3.48kg/m^2 and that of control group was 28 ± 2.56kg/m^2. Leptin levels among diabetic cases were 29.3± 19.3ng/ml and in non diabetic subjects it was 34.8 ±21.4 ng/ml. The difference in leptin levels between cases and control were statistically significant. Serum total cholesterol and triglycerides were significantly increased in diabetic group whereas LDL–C and HDL-C were statistically not significant as compared to control group.

Table 1: Anthropometric and physiological variables of diabetic and non diabetic subjects.

Variable	Non diabetic control	diabetic patients	P value*
Number	40	40	
Age(yrs)	42 ± 3.8	48 ± 12.03	0.04
BMI(kg/m^2)	28 ± 2.56	32 ± 3.48	0.01

Student t- test were applied for comparison
***p value <0.05 is statistically significant**

Table 2: Comparison of biochemical measurements in diabetics and non diabetics.

Variable	**Non diabetic control**	**diabetic patients**	**P value***
Leptin(ng/ml)	34.8 ±21.4	29.3± 19.3	0.001
TC(mg/dl)	190.3± 38.12	204.07± 44.89	0.003
TG(mg/dl)	178± 43.3	194.04±48.6	0.001
HDL-C(mg/dl)	40± 5.7	34± 4.3	0.06
LDL-C(mg/dl)	103±42.21	112±44.23	0.08
Fasting blood glucose(mg/dl)	92± 12.4	148± 52.6	0.001

Student t- test were applied for comparison
***p value <0.05 is statistically significant**

Discussion

We report lower circulating leptin levels in the diabetic subjects from an urban population of Indore M.P. India. These finding are consistent with those studies reporting lower serum leptin in subjects with diabetes in Caucasian and non-Caucasians populations [13 ,14, 15,16] but different from those reporting similar or higher serum leptin concentrations in subjects with diabetes [17-19].

A possible explanation of lower leptin levels in diabetic subjects is altered body fat distribution in diabetes. Subjects with diabetes have increased visceral fat and less subcutaneous fat [20]. Visceral fat produces less leptin than subcutaneous fat [20, 21]. Subjects with diabetes, therefore, would be expected to have lower circulating leptin than weight-matched controls as reported in this and other studies [13].

Relative insulin deficiency in type 2 diabetes may offer an alternative explanation for the lower leptin levels in diabetic subjects, since insulin is important stimulator of leptin production [22-25]. This also suggests that leptin may be a marker of insulin secretion rather than insulin sensitivity. In this study, females had higher serum leptin concentrations than males, but this did not reach statistical significance in diabetic subjects. The higher leptin levels in females than in males have previously been reported in population studies [26] and are probably due to gender differences in body fat distribution. Subcutaneous fat produces more leptin than visceral fat [20, 21]. Women, carry most of their higher body fat content subcutaneously, whereas men carry most of their lower body fat content viscerally [27,28]. The higher subcutaneous fat content in women would, therefore, explain their higher serum leptin concentrations. It however, has also been suggested that the gender differences in serum leptin may be related to the differences in sex hormones [27].

Limitation of study: The study had some limitations as did not include anthropometric data like waist circumferences and waist/hip ratio. It also did not homologize the diabetics in view of glycemic control and various treatment modalities. As the study was hospital based, the results can't be generalize to the community. Future studies with larger sample size having both sexes along with quantification of body fat content are needed to understand the role of leptin in detail in local population.

Conclusions

In summary, we reported that women and men with type 2 diabetes mellitus have lower serum leptin than nondiabetic controls. It remains to be established whether the lower leptin levels in subjects with diabetes are explained by altered fat distribution or relative insulin deficiency or both. Further studies are needed to

prospectively evaluate the relationship between reported factors and leptin levels among various groups of Indian population. Low leptin levels are associated with type 2 diabetes mellitus independent of changes in BMI.

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References

1. Rosenbaum M, Leibel RL. The role of leptin in human physiology. N Engl J Med 1999; Sep 16; 341 (12): 913-5.

2. Kraegen EW, Cooney GJ, Ye JM, Thompson AL, Furler SM. The role of lipids in the pathogenesis of muscle insulin resistance and β-cell failure in type II diabetes and obesity. Exp Clin Endocrinol Diabetes 2001;109(Suppl-2):S189–S201.

3. Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med. 1996 Feb 1; 334(5):292-5.

4. Sivitz WL, Walsh SA, Morgan DA, Thomas MJ, Haynes WG. Effects of leptin on insulin sensitivity in normal rats. Endocrinol 1997; Aug: 138(8):3395–401.

5. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Wnters D, Boone T, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995; Jul 28;269(5223):540–3.

6. Haffner SM, Stern MP, Miettinen H, Wei M, Gingerich RL. Leptin concentrations in diabetic and non-diabetic Mexican- Americans. Diabetes 1996; Jun; 45(6):822–4.

7. Schwartz MW, Prigeon RL, Kahn SE, Nicolson M, Moore J, Morawiecki A, et al. Evidence that plasma leptin and insulin levels are associated body adiposity via different mechanisms. Diabetes Care 1997; Sep; 20(9):1476–81.

8. Burtis CA,Ashwood ER,Bruns DE.Tietz textbook of clinical chemistry and molecular diagnostics;5th ed. Philadelphia,PA: Elsevier 2012:1431.

9. Burtis CA,Ashwood ER,Bruns DE.Tietz textbook of clinical chemistry and molecular diagnostics;5th ed. Philadelphia,PA: Elsevier 2012:771-772.

10. Burtis CA,Ashwood ER,Bruns DE.Tietz textbook of clinical chemistry and molecular diagnostics;5th ed.Philadelphia,PA: Elsevier 2012:772-773.

11. Burtis CA, Ashwood ER,Bruns DE.Tietz textbook of clinical chemistry and molecular diagnostics;5th ed. Philadelphia,PA: Elsevier 2012:773-775.

12. Burtis CA,Ashwood ER,Bruns DE.Tietz textbook of clinical chemistry and molecular diagnostics; 5th ed. Philadelphia, PA: Elsevier 2012:776.

13. Sandoval DA, Davis SN. Leptin: metabolic control and regulation. J Diabetes Complications 2003;Mar-Apr;17(2): 108-13.

14. Liew CF, Seah ES, Yeo KP, Lee KO, Wise SD. Lean, non-diabetic Asian Indians have decreased insulin sensitivity and insulin clearance, and raised leptin compared to Caucasians and Chinese subjects. Int J Obes Relat Metab Disord 2003; Jul; 27(7): 784-9.

15. Abdelgadir M, Elbagir M, Eltom M, Berne C, Ahren B. Reduced leptin concentrations in subjects with type 2 diabetes mellitus in Sudan. Metabolism 2002; Mar; 51(3):304-6.

16. Sayeed MA, Azad Khan AK, Mahtab H. Leptin is reduced in lean subjects with type 2 diabetes in Bangladesh. Diabetes Care 2003; Feb; 26(2): 547.

17. Buyukbese MA, Cetinkaya A, Kocabas R, Guven A, Tarakcioglu M. Leptin levels in obese women with and without type 2 diabetes mellitus. Mediators Inflamm 2004; Dec;13(5-6): 321-5.

18. Haque Z, Lakho R, Chundrigar T, Shahid KU, Mazahir I. Serum leptin levels correlate with leukocyte count in diabetic persons. Ann Abbasi Shaheed Hosp Karachi Med Dent Coll 2004; 9: 510-6.

19. Misra A, Arora N, Mondal S, Pandey RM, Jailkani B, Peshin S, et al. Relation between plasma leptin and anthropometric and metabolic covariates in lean and obese diabetic and hyperlipidaemic Asian Northern Indian subjects. Diabetes Nutr Metab2001;14(1)::18-26.

20. Al-Daghri N, Al-Rubean K, Bartlett WA, Al- Attas O, Jones AF, Kumar S. Serum leptin is elevated in Saudi Arabian patients with metabolic syndrome and coronary artery disease. Diabet Med 2003; Oct; 20(10): 832-7.

21. Montague CT, O'Rahilly S. The perils of portliness: causes and consequences of visceral adiposity. Diabetes 2000; Jun; 49(6): 883-8.

22. Cnop M, Landchild MJ, Vidal J. The concurrent accumulation of intra-abdominal and subcutaneous fat explains the association between insulin resistance and plasma leptin concentrations: distinct metabolic effects of two fat compartments. Diabetes 2002; Apr; 51(4): 1005-15.

23. Ahren B, Larsson H, Wilmhansson C, Nasman B, Olsson T. Regulation of circulating leptin in humans. Endocrine 1997; Aug;7(1): 1–8.

24. Mohamed-Ali V, Pinkney JH, Panahloo A, Goodrick S, Coppack SW, Yudkin JS. Relationships between plasma leptin and insulin concentrations, but not insulin resistance, in non-insulin-dependent (type 2) diabetes mellitus. Diabet Med 1997; May; 14(5): 376-80.

25. Haffner SM, Miettinen H, Mykkanen L, Stern MP. Leptin concentrations are associated with higher proinsulin and insulin concentrations but a lower proinsulin/insulin ratio in non- diabetic subjects. Int J Obes Relat Metab Disord 1998; Sep;22(9): 899-905.

26. Panarotto D, Ardilouze JL, Tessier D, Maheux P. The degree of hyperinsulinemia and impaired glucose tolerance predicts plasma leptin concentrations in women only: a new exploratory paradigm. Metabolism 2000; Aug; 49(8): 1055-62.

27. Van Gaal LF, Wauters MA, Mertens IL, Considine RV, De Leeuw IH. Clinical endocrinology of human leptin. Int J Obes Relat Metab Disord 1999; Feb;23(Suppl.1): 29-36.

28. Blaak E. Gender differences in fat metabolism. Curr Opin Clin Nutr Metab Care 2001; Nov;4(6): 499-502.

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