Research Article

Leptin and C-peptide Level in North Indians in Relation to Subjects Underlying Type Two Diabetes Mellitus

Jyoti Jain[1]*, Rati Mathur[1], Mansi Jha[1], Sachdev Yadav[2]
[1]Department of Biochemistry, SMS Medical College and Hospital, Jaipur, Rajasthan, India.
[2]Assistant Professor, Department of Pharmacy, Banasthali University, Rajasthan, India.
*Corresponding author's E-mail: jyotijain_1983@yahoo.com

ABSTRACT

Leptin is an adipocyte secreting hormone which regulate appetite and body weight more specially body fat stores. Its concentration differs in various metabolic diseases, so the purpose of this study was to measure Leptin and C-peptide level in diabetic and healthy subjects and find its association with anthropometry measurement and various metabolic indices. The study was conducted in SMS hospital Jaipur. 150 type two diabetes mellitus and 150 healthy control age and sex matched were recruited for this study. BMI, weight, height, waist circumference, hip circumference were measured and biochemical parameters fasting blood glucose, insulin, lipids, leptin and C-peptide were measured. Total 150 control subjects (84 male and 66 female) and 150 diabetic subjects (86 male and 64 female) with the mean age of (44.1±10.4) y for control subject and (44.12±8.50) y for diabetic subjects were recruited. BMI, waist circumference, hip circumference were found significantly higher (p<0.05) in diabetic subjects than control group. Biochemical parameters triglycerides, total cholesterol, insulin and C-peptide were found significantly higher (P<.05) in diabetic group whereas serum leptin concentration was found significantly lower (p<.05) in diabetic subjects (11.54±5.47) than control subjects (14.44±3.44). Serum leptin concentration was found significantly higher in women than men in both control and diabetic subjects. The serum leptin was significantly correlated with BMI, Hip circumference, waist circumference, Insulin, HOMA-IR and C-peptide in both control and diabetic subjects. The results of the study shows that Serum leptin concentration decreased in diabetic subjects and leptin correlated positively with BMI, hip circumference and waist circumference. It was also correlated with insulin, HOMA-IR and C-peptide. Serum C-peptide concentration increased in diabetic subjects.

Keywords: Leptin, C-peptide, Diabetes, Insulin, HOMA-IR.

INTRODUCTION

In the present scenario the prevalence of chronic, non-communicable diseases is increasing day by day. About 18 million people die every year from cardiovascular disease, for which diabetes and hypertension are major predisposing factors[1]. It is estimated that diabetes affects about 150 million people worldwide, and this figure is expected to be doubled in the next 20 years[2]. T2DM is a complex heterogeneous group of metabolic conditions characterized by increased levels of blood glucose due to impairment in insulin action and insulin secretion[3]. Insulin resistance refers to suppressed or delayed responses to insulin. There are three defects in the onset of hyperglycemia in T2DM: increased hepatic glucose production, diminished insulin secretion, and impaired insulin action[4]. The adipose tissue not only releases free fatty acids but also hormones and cytokines such as leptin, adiponectin, resistin, TNF-alpha etc. These hormone and cytokines modify insulin action. Hence, the role of obesity in the generation of insulin resistance and subsequently leading to impaired glucose tolerance as well as overt type 2 diabetes mellitus has been established[5]. Low circulating levels of insulin is compelling for the anti-diabetic actions of insulin[6]. The two hormones namely leptin and insulin are producing the respective adipose tissue signalling by changing the whole dynamics of energy homeostasis[7]. Asian Indians are particularly susceptible to develop diabetes mellitus[8]. It is well established that leptin impairs insulin signalling and

confers insulin resistance. Leptin replacement in hypoleptinemic, lipoatrophic patients, however, improved glucose tolerance and decreased serum insulin concentrations[9, 10]. This clearly suggests that leptin may possess insulin sensitizing properties. Leptin is a crucial hormone in the regulation of body weight, more specifically of body fat stores[11, 12]. It is secreted by white adipose cells, and is directly related to body fat content. There is considerable inter individual variation in plasma leptin concentrations among individuals with comparable degrees of obesity, suggesting that other factors are involved in the regulation of leptin production. Leptin modulates immune system, reproduction, angiogenesis and lipolysis[13]. Centrally, leptin is an essential component of the feedback circuitry that integrates energy homeostasis, primarily by modulating the hypothalamic peptidergic network involved in energy intake and expenditure[14]. Leptin improves glucose tolerance and increases the expression of key enzymes, there by influencing the rate of gluconeogenesis and glycogenolysis via melancortin-dependent and independent pathways[15]. Leptin is a critical signaling molecule in the hypothalamus influencing appetite and satiety. There is a positive correlation between the circulating levels of leptin and adipocyte number and size[16]. Leptin levels have been found to be correlated with fasting insulin levels, subcutaneous adipose tissue and BMI, but not with glucose disposal in Asian-Indian men[17]. Hyperleptinaemia has been advocated as a

component of the insulin resistance syndrome, and the insulin–leptin axis may play a coordinating role in this syndrome[18]. The great interest in C-peptide is due to the limitations of the use of serum insulin as a measure of insulin secretion. After its secretion in to the portal vein, insulin passes through the liver where approximately 50% of the insulin delivered is extracted[19]. C-peptide is produced by a series of enzymatic cleavages of the precursor molecules preproinsulin and proinsulin. Preproinsulin, a precursor of proinsulin, is produced in the endoplasmic reticulum of pancreatic β-cells in response to elevated blood glucose levels in healthy individuals; it is then cleaved by microsomal enzymes into proinsulin. C-peptide is cleared by the kidney and has a half-life of about 20 to 30 minutes compared to insulin which is cleared through the liver and has a half-life of about 3 to 5 minutes[20].Because C peptide is secreted from islet cells into the circulation in equimolar concentrations with insulin and is not extracted by the liver, many investigators have used C peptide levels as a biomarker of β-cell function[21].

SUBJECTS AND METHODS

Subjects

Two group of subjects were recruited (1) Type 2 diabetes mellitus which included 150 patients visiting endocrinology OPD of SMS hospital Jaipur age 25 -65. Patients with secondary Diabetes mellitus, other endocrinopathies, Ischemic heart disease, Clinical condition that influence insulin resistance, and patients on drugs like steroids, pioglitazone, metformin and any medication known to affect body composition were excluded from the study. (2) Second group comprises 150 patients, age and sex matched healthy control group. The study protocol was approved by Ethics Committee SMS hospital Jaipur. Anthropometric measurements were recorded for all patients. Height, weight, waist and hip circumference were taken by standard procedures. Waist to Hip ratio (WHR) was calculated as Waist circumference (cm) divided by Hip circumference (cm). Body mass index (BMI) was calculated using the formula: weight (kg) divided by height in meters squared.

Biochemical assay

Blood samples were obtained by vein puncture after overnight fasting (minimum 12 hours fasting) to estimate the following biomarker: (1) Serum leptin and C-peptide Concentrations enzyme- linked immunoassay (i) Avi Bion Human Leptin ElISA Kit; Orgenium laboratories business Unit Finland; (ii) Dia Metra C-peptide ELISA Kit. (2) Serum insulin was estimated by chemiluminescent immunometric assay using Immulite 2000 machine. (3) Fasting Blood glucose, Serum lipid profile (Total cholesterol, Triglycerides, HDL,) were measured using commercial available kits (Recombigen, Anamol, Becon, Logotech) on fully automated analyzer (AU400/Kopran). LDL was calculated by fire wield formula. Serum was stored at -20°C for analysis of leptin and C-peptide.

Indexes

The BMI was calculated as body weight (kg)/height (m)^2. Waist to Hip ratio (WHR) was calculated as Waist circumference (cm) divided by Hip circumference (cm). The homeostasis model assessment index HOMA-IR = (fasting plasma glucose (mg/dl) x insulin (μU/ml)/405.) for insulin resistance was calculated.

Statistical analysis

Statistical analysis was performed using IBM Statistical Package for Social Sciences SPSS for windows (version 19, Chicago, IL, USA). Means and standard deviations (SD) were calculated for all parameters. The independent sample t-test was used to compare the means of different variables in the two groups. In addition, the Pearson correlation coefficient (r) was used for correlation analysis of leptin and C-peptide in Diabetic and Control Group. For all statistically assessment a value of $p < 0.05$ was considered significant.

RESULTS

Anthropometry, biochemical parameters, leptin and C-peptide in Control group and Diabetic group are summarized in Table 1. The mean age of control group was 44.1±10.4 years and that of diabetic group 44.12±8.5 years. Significant differences in BMI, waist circumference, hip circumference, triglycerides, cholesterol and HDL, were found between the two groups. The fasting blood glucose and HOMA IR of diabetic group were significantly higher whereas the leptin concentrations were found lower (11.54±5.47) than the control group (14.44±3.44) and C-peptide was found significantly higher in diabetic patients (3.13±1.02) than the control group (2.18±1.41) when compared with group 1 via independent t – test.

Table1: Anthropometry and biochemical parameter of the study subjects (mean±SD)

Variables	Total		
	Control	Diabetic	p
N (Male/Female)	150	150	
Age(yrs.)	44.1±10.4	44.12±8.5	NS
BMI(Kg/m^2)	23.45±2.4	26.52±4.39	0.000
WC(Cm)	82.28±12.67	94.37±11.08	0.000
HC(Cm)	87.96±12.79	98.13±11.7	0.000
WHR	0.93±0.096	0.96±.08	0.003
TG (mg/dl)	119.39±50.01	156.18±73.51	0.000
Cholesterol (mg/dl)	180.47±39.86	191.57±41.60	0.029
HDL(mg/dl)	47.47±7.15	43.74±5.3	0.000
LDL	109.40±34.58	116.56±37.90	NS
HOMAIR	1.05±0.84	2.6±2.1	0.000
Insulin(μU/ml)	4.66±3.60	6.5±5.15	0.0007
Glucose(mg/dl)	90.9±9.78	170.03±56.12	0.000
Leptin(ng/ml)	14.44±3.44	11.54±5.47	0.000
C-Peptide (ng/ml)	1.72±0.88	3.13±1.02	0.000

BMI= Body Mass Index; WC= Waist Circumference; HC= Hip Circumference; WHR= Waist to hip ratio; TG= Triglycerides; HDL= High

density lipoprotein; LDL= Low Density lipoprotein. P<0.05 is significantly different.

The comparative study of diabetic male and diabetic female and control male and control female was shown in Table 2. No significant difference was found in Anthropometry measurement in both diabetic and Control male, female except Waist to hip ratio which showed significant difference in both diabetic male and diabetic female and control male and control female. In biochemical parameters HDL showed significant difference in control male and female but no significant difference was found in diabetic male and female. Serum leptin level was found significantly higher in female subjects in both diabetic and control.

Table 2: Gender based Anthropometry and biochemical parameter of the study subjects (mean±SD)

Variables	Diabetic male	Diabetic Female	P	Control male	Control female	p
N	86	64		84	66	
Age(yrs.)	45.71±8.42	45.78±9.61	NS	44.91±11.2	43.08±9.4	NS
BMI(Kg/m^2)	26.60±4.39	26.40±4.43	NS	23.49±2.42	23.39±2.48	NS
WC(Cm)	95.48±11.07	92.89±11.01	NS	83±12.65	81.38±12.73	NS
HC(Cm)	97.55±10.89	98.90±12.9	NS	86.83±11.59	89.37±14.11	NS
WHR	0.98±.09	0.94±.061	0.002	0.95±0.09	0.91±0.09	0.007
TG (mg/dl)	160.09±76.06	150.94±70.18	NS	125.59±49.56	111.71±49.87	NS
Cholesterol (mg/dl)	201.08±34.78	194.72± 44.2	NS	178.24±42.99	183.23±35.73	NS
HDL(mg/dl)	44.06±5.58	43.32±5.1	NS	45.92±7.36	49.39±6.4	0.002
LDL	113.28±37.22	120.97±38.65	NS	106.79±36.93	112.64±31.39	NS
HOMAIR	2.5±1.94	2.84±2.50	NS	1.09±0.77	1.003±0.92	NS
Insulin	6.4±5.45	6.6± 5.56	NS	4.76±3.27	4.54±4.01	NS
Glucose(mg/dl)	165.38±49.80	176.28 ± 63.51	NS	92.14±9.53	89.57±9.8	NS
leptin(ng/ml)	10.55±5.07	12.88±5.73	0.009	13.35±2.20	15.80±4.16	0.0001
C-peptide (ng/ml)	3.1±1.01	3.1± 1.05	NS	1.66±0.85	1.79±0.92	NS

P<0.05 is significantly Significant. NS= Not significant

There was a strongly significant positive correlation of Leptin with BMI (r=.828, p=.0001) hip circumference (r=.254, p=.01), and waist circumference (r=.320, p=.0001) in control and (r=.756, p=.0001), (r= .514, p=.0001), (r=.522, p=.0001) in diabetic group was found. There was No correlation found with waist to hip ratio in both diabetic and control group as shown in Table 3.

Table 3: Correlation of leptin with Anthropometry in diabetic and control group

Variables	Control		Diabetic	
	r	P	r	p
BMI	0.828	0.01	0.756	0.000
WC	0.254	0.001	0.514	0.000
HC	0.320	0.000	0.522	0.000
WHR	-0.06	0.465	-0.016	0.843

In metabolic variable leptin show significant positive correlation with HOMA-IR (r=.197, p=.016) Insulin (r=.277, p=.001) and C-peptide(r=.652, p=.000) in diabetic group. Leptin also showed significant positive correlation with HOMA-IR(r= .169, p=.038) and Insulin (r=.175, p=.032) in control group although it is not as strong as showed in Diabetic group. Leptin did not show any correlation with HDL and LDL in both Control and Diabetic group as shown in Table 4.

Table 4: Correlation of leptin with biochemical parameter in diabetic and control group

Variables	Control		Diabetic	
	r	P	r	p
HDL	0.146	0.07	0.09	0.275
LDL	0.104	0.205	0.110	0.179
HOMA IR	0.169	0.038	0.197	0.016
Insulin	0.175	0.032	0.277	0.001
C-peptide	0.699	0.00	0.652	0.000

DISCUSSION

In This present study we demonstrated serum leptin and C-peptide concentration in diabetic patients against control subjects. It was found that serum leptin concentration was significantly lower in diabetic patients and it also showed a positive correlation with body mass index in both control and diabetic subjects. Leptin is an adipose –derived hormone that has been shown to be involved in pathways influencing the risk of cardiovascular disease and diabetes[22]. It has been suggested that the association between plasma leptin and diabetes may be a manifestation of an underlying leptin resistance mediated by obesity[23]. Our results consistent with previous study by ching chu chen, et al. Roden et al.[24] found that both type 1 and type 2 diabetic patients in good metabolic control display lower basal plasma leptin concentrations

than non-diabetic humans suggesting a potential role of glycaemia for leptin secretion.[25] However, the regulation of serum leptin levels in humans is not well understood. Some authors reported that serum leptin level increases as body fat mass increases[26, 27]. Obese people have increased level of leptin and it could be due to resistant action of leptin on inhibition of food intake and increase in energy expenditure[28]. Rohner-Jeanrenaud discussed that if there is a defect in the leptin pathway in human obesity, it could be due either to an inability of the leptin to appropriately enter the central nervous system or to a post-receptor defect in the subsequent leptin signaling cascade. It is likely that the transmission of the signal will involve many other molecules in the central nervous system. Particularly prominent studies have focused on neuropeptide Y and the proopiomelanocortin (POMC) (MSH precursor) pathway[29]. Clement K et al. reported that plasma leptin was significantly reduced in the diabetic individual in morbidly obese subjects who were in poor metabolic control[30]. Our study reported that women have relatively high leptin concentration than in men and this study is supported by Hellstroem; provides evidence that gender differences in circulating leptin levels are caused by two different mechanisms: a larger adipose tissue mass and a higher production rate of leptin per unit mass of adipose tissue in women than in men[31]. Luukkaa et .al. found the strong evidence that the testosterone is probably one of the most important factors contributing to the lower serum leptin levels in men compared with women[32]. Wabitsch reported that testosterone and its active metabolite dihydrotestosterone were able to suppress leptin secretion and leptin messenger ribonucleic acid in primary culture of human adipocytes, suggesting a direct effect of testosterone at the level of adipocytes[33] our study reported significant positive correlation of leptin with insulin, HOMAIR and C-peptide. Our study supported by Radka Lichnovska[34] and discussed that leptin could be a mechanism by which increased adiposity increases insulin resistance. Leptin antagonizes insulin signaling in hepatoma cells, decreasing insulin-induced tyrosine phosphorylation of IRS-1, a step leading to many of the metabolic actions of insulin (glucose transport, kinase pathway). Leptin also antagonizes the ability of insulin to decrease mRNA encoding PEPCK, the enzyme catalyzing the rate-limiting step in gluconeogenesis[35]. In our study leptin significantly correlated with waist and hip circumference and this result is consistent with the finding of De courtesan[36]. The leptin mRNA levels and secretion rates are higher in subcutaneous than in visceral adipose tissue and positive correlation between leptin and waist circumference may, therefore, reflect the contribution of subcutaneous abdominal fat mass and may link leptin with central obesity[37]. In our study C-peptide was that significantly increased in Diabetic patients. The same findings was reported by bilal b Abdullah[38] and concluded that the fasting c-peptide levels are useful in type 2 diabetic patients with poor glycaemic control to assess the endogenous insulin reserve and to

alter the modality of treatment. Insulin is secreted as pro-insulin and subsequently cleaved into insulin and C-peptide[39]. C-peptide is a marker of pancreatic insulin synthesis, and several epidemiologic studies have utilized C-peptide as an alternate biomarker to insulin because it has a longer half-life than insulin and therefore is more stable[39]. Our result did not show any correlation of leptin with triglyceride, Total cholesterol, LDL cholesterol, and HDL cholesterol in both control and diabetic patients. The same result was reported by ali al-Sultan (2006) in normal weight and obese Saudi adults[40].

CONCLUSION

In conclusion our findings reported that serum leptin level decreased in type two diabetes mellitus subjects. The women have relatively high concentration of serum leptin level than in men. Leptin level increased with body mass index. Our findings of a strong association between leptin and insulin sensitivity suggest an important role for leptin in human metabolism. C-peptide increased in type two diabetes mellitus and it is positively correlated with leptin. C-peptide is also a marker of metabolic disease.

REFERENCES

1. Haslam DW, James WP, Obesity, Lancet 2005, 366: 1197-1209.

2. Zimmet P, Alberti KG, Shaw J, Global and societal implication of diabetes epidemic, Nature 2001, 414:782-787.

3. Das SK, Elbein SC, The genetic basis of type 2 diabetes, Cell science. 2006, 2: 100–131.

4. Stumvoll M, Goldstein BJ, Van Haeften TW, Type 2 diabetes: Principles of pathogenesis and therapy, Lancet 2005, 365: 1333–1346.

5. Małecki MT, Obesity--insulin resistance--type 2 diabetes mellitus. Kardiologia Polska, 2006, 64:61-6.

6. Kalra SP, Central leptin gene therapy ameliorates diabetes type 1 and 2 through two independent hypothalamic relays; A benefit beyond weight and appetite regulation. Peptides 2009, 30: 1957–1963.

7. Scherer T, Buettner C, Yin and Yang of hypothalamic insulin and leptin signaling in regulating white adipose tissue metabolism. Reviews in Endocrine & Metabolic Disorders, 2011, 12: 235-43.

8. King H, Aubert R E, Herman W H, Diab. Care, 21:1998, 1414–1431.

9. Oral EA, Simha V, Ruiz E, Leptin-replacement therapy for lipodystrophy, N Engl J Med. 346:2002, 570–8.

10. Petersen KF, Oral EA, Dufour S, Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy, J Clin Invest, 109:2002, 1345–50.

11. Considine RV, Sinha MK, Heiman ML, Kriauciuna, A, Stephens TW, Serum immunoreactive-leptin concentrations in normalweight and obese humans, N Engl J Med, 334:1996, 292–295.

12. Ostlund RE, Yang JW, Klein S, Gingerich R, Relation between plasma leptin and body fat, gender, diet, age and metabolic covariates, J Clin Endocrinol Metab, 81: 1996, 3909–3913.

13. Kalra SP, Central leptin insufficiency syndrome: An interactive etiology for obesity, metabolic and neural diseases and for

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ISSN 0976 – 044X

designing new therapeutic interventions. Peptides, 29:2008, 127–38.

14. Kalra SP, Disruption in the leptin-NPY link underlies the pandemic of diabetes and metabolic syndrome: New therapeutic approaches. Nutrition. 24:2008, 820–26.

15. Gutierrez-Juarez R, Obici S, Rossetti L, Melanocortin-independent effects of leptin on hepatic glucose fluxes. The Journal of Biological Chemistry, 279:2004,704–715.

16. Skurk T, Alberti-Huber C, Herder C, Hauner H, Relationship between adipocyte size and adipokine expression and secretion. Journal of Clinical Endocrinology and Metabolism, 92:2007, 1023–1033.

17. Banerji MA, Faridi N, Atluri R, Chaiken RL, Lebovitz HE, Body composition, visceral fat, leptin, and insulin resistance in Asian Indians Men, J Clin Endocrinol Metab, 84: 1999, 137–144.

18. Leyva F, Godsland IF, Ghatei M, Proudler AJ, Aldis S, Hyperleptinaemia as a component of a metabolic syndrome of cardiovascular risk, Arterioscler Thromb Vasc Biol, 18:1998, 928–933.

19. Ferranini E, Cobelli C. The kinetics of insulin in man. Role of the liver, Diabetes Metab Rev, 3:1987, 365-97.

20. Marques RG, Fontaine MJ, Rogers J, C-peptide: much more than a byproduct of insulin biosynthesis, Pancreas, 29(3): 2004, 231-8.

21. Faber OK, Binder C, C-peptide: an index of insulin secretion, Diabetes Metab Rev, 2:1986, 331-45.

22. Wannamethee SG, Tchernova J, Whincup P, Lowe GDO, Kelley A, Rumley A, Wallace AM, Sattar N, Plasma leptin: Associations with metabolic, inflammatory and haemostatic risk factors for cardiovascular disease, Atherosclerosis, 191:2007, 418–426.

23. Steinberg GR, Parolin ML, Heigenhauser GJ, Dyck DJ, Leptin increases FA oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance, A J Physiol Endocrinol Metab, 283:2002, E187–E192

24. Ching-Chu Chen, Tsai-Chung Li1, Chwen-Tzuei Chang, Chih-Ming Chien, Rong-Hshing Chen, Tzu-Yuan Wang Lower Serum Leptin Concentrations in Female Subjects with Type 2 Diabetes Mellitus, Mid Taiwan J Med, 2003, 8:8-12.

25. M Rodenl, C Ludwig, P Nowotny, B Schneider, M Clodil, H Vierhapper, A Roden and W Waldhäus Relative hypoleptinemia in patients with type 1and type 2 diabetes mellitus. International Journal of Obesity, 24, 2000, 976-981.

26. Campfield LA, Smith FJ, Burn P, The ob protein (leptin) pathway—a link between adipose tissue mass and central neural networks, Horm Metab Res, 28;1996, 619–632.

27. Rosenbaum M, Nicolson M, Hirsch J, Murphy E, Chu F, Leibel RL, Effects of weight change on plasma leptin concentration, and energy expenditure, J Clin Endocrinol Metab, 82:1997, 3647–3654.

28. F-Xavier, Pi-Sunyer, Blandine, Laferre Re, Louis J. Aronne, and George A. Bray ,Metabolic Abnormalities and the Role of Leptin in Human Obesity. Journal of Clinical Endocrinology and Metabolism, 84;1999, 1-7.

29. Rohner-Jeanrenaud F, Cusin I, Sainsbury A, Zakrzewska KE, Jeanrenaud B, The loop system between neuropeptide Y and leptin in normal and obese rodents, Horm Metab Res. 28:1996, 642– 648.

30. Clement K, Lahlou N, Ruiz 1, Hager 1, Bougneres P, Basdevant A, Guygrand B, Froguel P,Association of poorly controlled diabetes with low serum leptin in morbid obesity, Int J Obes, 21:1997, 556-561.

31. Hellstroèm L, Wahrenberg H, Hruska K , Reynisdottir S, Arner P, Mechanisms behind gender differences in circulating leptin levels, Journal of Internal Medicine, 247:2000, 457-462

32. Virve L, Ullamari P, Ilpo H, Aapo L, Reijo T, Jaakko T, Markku K, Risto H, Inverse Correlation between Serum Testosterone and Leptin in Men, Journal of Clinical Endocrinology and Metabolism, 83;1998, 3243-3246.

33. Wabitsch M, Blum WF, Muche R, Contribution of androgens to the gender difference in leptin production in obese children and adolescents, J Clin Invest, 100;1997, 808–813.

34. Radka L, Simona G, Rudolf C, Jiři H, Serum leptin in the development of insulin resistance and other disorders in the metabolic syndrome, Biomed. Papers, 149(1);2005, 119–126.

35. Taylor SI, Barr V, Reitman M, Does leptin contribute to diabetes caused by obesity, Science, 274:1996, 1151–1152.

36. De Courten M, Zimmet P, Hodge A, Collins V, Nicolson M, Staten M, Hyperleptenemia; the missing link in metabolic syndrome, Diabeties Med, 14:1997, 200–8.

37. Minocci A, Savia G, Lucantoni R, Berselli ME, Tagliaferri M, Calo G, Leptin plasma concentrations are dependent on body fat distribution in obese patients, Int J Obes Relat Metab Disord, 24:2000, 1139–44.

38. Bilal bin abdullah, Basanagouda S Patil and A, Thaseen Significance of C – Peptide in Type 2 Diabetics - A Study in the North Karnataka Population of India, Al Ame en J Med Sci, 3(1):2010, 65 -78

39. Hovorka R, Jones RH, How to measure insulin secretion, Diabetes Metab Rev, 1994, 10:91–117.

40. Ali I. Al-Sultan, Abdulmohsen H. Al.Eiq, Leptin levels in normal weight and obese Saudi adults, Journal of family and community medicine, 13:2006, 3.