Original article:

Leptin level alterationamong non-obese type 2 diabetic male & female – How is it related to insulin resistance & what possible role it has in development of long term diabetic complications?

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Abstract

Purpose: Leptin is hormone secreted by adipocytes that plays a significant role in numerous metabolic processes including development of type 2 diabetes. In this study, we had intended to understand the relationship of leptin levels with various insulin resistance indices among non-obese diabetic patients as well as its role in long term diabetic complications.

Materials & Methods: In this case control study, we classified subjects with respect to the presence or absence of long term diabetic complications. Routine blood and urine biochemistry, HbA1C, fasting insulin and leptin levels were measured. Insulin resistance indices were calculated from those measured values.

Results: Results revealed that that the prevalence of long term diabetic complications were maximum when there was a significant reduction in leptin level irrespective of sex of patient. Leptin levels were positively influenced by fasting insulin levels, HOMA-IR and HOMA-β in both male and female diabetics. They were negatively been influenced by QUICKI and Glucose-Insulin ratio among both genders. Decreased leptin levels in the presence of diabetic complications may primarily be due to drastic reduction in insulin secretion because of absolute loss of pancreatic beta cell functioning.

Conclusion: Besides age, fat mass and gender, the main determinant of leptin levels among diabetic patients was insulin secretion and high degree of insulin resistance seem to contribute significantly to leptin level raise. Leptin level decreases drastically in the presence of long term diabetic complications though the decrease has possibly no influence with those developments.

Key words: Diabetes mellitus, Leptin, HOMA-IR, HOMA-β, QUICKI, Glucose-Insulin ratio

Introduction:

Leptin, a direct product of adipose cell is a satiety hormone, which regulates food intake and energy utilization [1,2]. While these facts are well established, growing evidence suggest that leptin is also critical for glycemic control. It has been independently associated with development of insulin resistance and ultimately type 2 diabetes mellitus. It is also being suggested that the association between plasma leptin and diabetes may be a manifestation of underlying leptin resistance mediated obesity [3]. This study was intended to understand the relationship of leptin levels with various insulin resistance indices among non-obese diabetic patients as well as its role in long term diabetic complications.

Materials and Methods:

This study was conducted for a period of one year from Jan 2018 to Dec 2018 at a rural tertiary care Government hospital in southern India after getting approval from the Institutional Scientific and Ethical committee. The study has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its latter

amendments. Our case control study included 78 diabetic male patients and 70 diabetic female patients between the age group of 35 to 55 years. Patients having clinically confirmed long term diabetic complications were regarded as cases and patients without diabetic complications were considered controls. All patients included in our study were non-obese diabetic patients (Body Mass Index (BMI) 18.5-22.9 kg/m^2) of both the sexes. Patients with clinical evidence of diabetic nephropathy, diabetic retinopathy & diabetic neuropathy/ diabetic foot ulcers, diabetic related cardiovascular complications or any vessel related complications were included as patients having diabetic complications. Underweight/ overweight or obese individuals (BMI <18.5 and ≥23.0 kg/m^2), patients having extremes of age (< 35 years and >55 years), patients not clinically diagnosed with diabetes, patients having any chronic co-existing disease conditions along with diabetes, patients on insulin therapy, diabetic patients with previous history of any form of renal, cardiovascular, ophthalmic, peripheral vascular disease complications developed even before the onset of diabetes and critically ill patients were excluded from our study.

After explaining the nature of the study, written consent was obtained from all subjects before collecting blood and spot urinesamples. Spot urine samples were collected to measure Urine Microalbumin-Creatinine Ratio (UCMR) (represented as mg of microalbumin per gram of creatinine excreted). All samples for biochemical investigations were collected after patients exhibiting overnight fasting. Using standard measures of height and weight, BMI was measured using Quetelet's index (BMI = weight (kilograms)/height (metre^2)). Classification of patients according to their BMI was done according to the cut-offs set for the Asian population by WHO [4]. The study population of diabetic patients were stratified into two groups based on the presence or absence of diabetic complication by physical examination and investigation. Fundus examination was done by an ophthalmologist to rule out the presence of retinopathy. Serum urea, creatinine and urine microalbumin-creatinine ratio were done to know the presence of nephropathy. ECG/ Echo were taken to identify cardiovascular complication. Physical examination for foot ulcers and nerve conduction studies were done to confirm the presence of neuropathy. Percentage body fat was calculated using BMI [5].

Body fat percentage for adult males = 1.20 X BMI + 0.23 X age – 16.2 Body fat percentage for adult females = 1.20 X BMI + 0.23 X age – 5.4

All biochemical analysis in serum and urine were carried out in Dimension RxL max Integrated Chemistry system from Siemens healthineers. HbA1C measurement was carried out by ion exchange resin method using Glycated Hemoglobin kit from Biosystem**.** Serum leptin values were evaluated using sandwich ELISA method using leptin-ELISA kit from DIAsource.

The Insulin resistance indices used in our study are Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), Homeostatic Model Assessment for β-cell functioning index (HOMA-β), Qualitative Insulin Sensitivity Check Index (QUICKI), Glucose-Insulin Ratio (GI ratio) and Glucose-Leptin Ratio (GL ratio).

HOMA-IR was calculated using the formula (Glucose X Insulin)/405 where Glucose is represented as mg/dL and Insulin as μIU/mL [6].

HOMA- β was calculated using the formula (360 X Insulin)/Glucose – 63 where Glucose is represented as mg/dL and Insulin as μIU/mL [6].

QUICKI was calculated using the formula 1/ (log (glucose) + log (insulin)) where Glucose is represented as mg/dL and Insulin as μIU/mL [7].

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Statistical analysis

Data was analysed using SPSS software 16.0 version for windows. All values showing parametric distribution were presented as mean ± standard deviation (Mean ± SD). Non-parametric distribution values were presented as median ± interquartile range (IQR) between 25th and 75th percentile.Comparison between cases and controls were carried out by independent sample t-test in case of parametric distribution and Mann Whitney test in case of non-parametric distribution. Further, male and female subjects were divided into six groups according to their corresponding leptin percentiles measured (that is G<5=leptin value less than 5th percentile, G5-25=leptin value between 5th to 25th percentile, G25-50= leptin value between 25th to 50th percentile, G50-85=leptin value between 50th to 85th percentile, G85-95= leptin value between 85th to 95th percentile and G>95= leptin value more than 95th percentile) and various characteristics across them were compared by Kruskal-Wallis Test.Multiple linear regression analysis was performed to test the effect of independent variables on leptin levels among diabetic male & female. A 'p' value of less than 0.05 was considered statistically significant.

Results:

Out of 148 clinically confirmed diabetic patients, 78 (53%) were male, and remaining 70 (47%) were female. All patients selected were having BMI ranging from 18.5-22.9 kg/m^2 since this study was carried only among non-obese diabetic patients. Both male and female patients selected were further stratified into two subgroups, according to the presence or absence of clinically evident long term diabetic complications. Patients with clinical evidence of long term diabetic complications were deemed as 'case' and those without complications were regarded as 'control'.The comparison of general demographics, routine biochemical investigations and insulin resistance indices between cases and controls for both the sexes were explained in Table 1. Mean age, fasting/ postprandial blood glucose, creatinine and triglycerides were found to be increased among patients with long term diabetic complications, but were not significant at 5% level in both sexes. While mean HbA1C, median UCMR, QUICKI index, Glucose-Insulin Ratio, Glucose-Leptin Ratio were significantly increased among cases, mean fasting insulin levels, leptin levels, median HOMA-IR and HOMA- β values were found to be significantly decreasedamong them irrespective of their gender. Percentage body fat, Serum urea and HDL levels were found to have no change between those stratified groups.

Further, 78 male subjects and 70 female subjects were classified according to their corresponding leptin percentiles for comparing various characteristics across them, as shown in table 2 and table 3 respectively. These groups did not differ significantly in relation to age, BMI, fasting/ postprandial blood glucose, urea, creatinine, total cholesterol, triglycerides, HDL cholesterol and percentage body fat for both male and female, although the group with less than 50th percentile of leptin had higher median age and lower median BMI. The groupG<5 with lower leptin level (<5th percentile of leptin cut-off of 6.04 ng/mL for male and 7.66 ng/mL for female) presented with maximum prevalence of long term diabetic complications (100% Vs 0%), higher median UCMR (124mg/g vs 28 mg/g for male; 263 mg/g Vs 26 mg/g for female), lower median fasting insulin (5.75 µIU/mL vs 19.98 µIU/mL for male; 9.26 µIU/mL vs 25.38 µIU/mL for female), lower median insulin resistance index (HOMA-IR 2.29 vs 4.88 for male; 2.70 vs 8.11 for female), lower median β-cell function index (HOMA-β 21.13 vs 224.43 for male; 58.50 vs 135.65 for female), increased median qualitative insulin sensitivity check index (QUICKI 0.34 vs 0.30 for male; 0.33 vs 0.28 for female) and higher median GI ratio (24.77 vs 4.76 for male; 12.08 vs 5.15 vs female) when compared with G>95.

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Table 1: Sex stratified comparison of demographics, biochemical analytes and insulin resistance indices among diabetic patients with and without complications

	Male diabetic patients (n=78)			Female diabetic patients (N=70)		
	without complications	with complications	*p* value	without complications	with complications	*p* value
N (%)	39 (50)	39 (50)	NA	36 (51)	34 (49)	NA
Age (Years)	45±6	47±6	0.114	45±5	46±6	0.648
BMI (kg/m^2)	21.5±1.0	20.9±1.4	0.068	21.4±1.0	20.3±1.3	0.799
Glucose (Fasting) mg/dL	147±58	184±73	0.017*	158±67	165±72	0.672
Glucose (Postprandial) mg/dL	245±87	287±104	0.057	235±83	276±112	0.089
HbA$_{1C}$	7.7±0.6	8.3±0.9	0.003*	7.7±0.7	8.1±1.0	0.049*
Urea mg/dL	25±6	25±6	0.823	26±8	25±6	0.619
Creatinine mg/dL	0.72±0.14	0.75±0.14	0.252	0.76±0.16	0.79±0.15	0.343
Cholesterol (Total) mg/dL	185±37	182±47	0.715	176±46	191±43	0.178
Triglycerides mg/dL	128±46	142±57	0.224	123±61	140±67	0.257
HDL mg/dL	41±6	41±5	0.969	41±6	41±5	0.789
UMCR mg/g	28.1 (21.2,31.1)	64.1 (34.8,209.1)	<0.001*	25.2 (20.4,28.9)	49.6 (33.8,172.7)	<0.001*

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Insulin µIU/mL	16.39±2.41	10.54±2.27	<0.001*	22.07±2.18	13.13±3.50	<0.001*
Body at %	20±2	20±2	0.742	31±2	31±2	0.898
HOMA-IR	4.92 (4.30,7.29)	4.49 (3.1,6.25)	0.028*	7.58 (6.03,9.97)	4.99 (2.98,7.04)	<0.001*
HOMA-β	88.59 (50.1,149.59)	34.15 (22.32,57.49)	<0.001*	114.64 (58.92,169.5)	56.58 (35.32,97.15)	0.001*
QUICKI	0.30 (0.29,0.31)	0.31 (0.29,0.32)	0.028*	0.29 (0.28,0.30)	0.30 (0.29,0.32)	<0.001*
GI Ratio	8.27 (6.1,11.02)	15.47 (11.34,22.56)	<0.001*	5.90 (4.89,8.82)	11.30 (8.78,16.93)	<0.001*
GL Ratio	10.67 (7.73,14.37)	20.35 (14.79,29.88)	<0.001*	7.48 (6.12,11.4)	14.74 (11.35,22.32)	<0.001*
Leptin ng/mL	12.9±1.9	8.3±1.8	<0.001*	17.4±1.7	10.4±2.7	<0.001*

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Parametric distributions are presented as mean ±Standard Deviation; Non-parametric distributions are presented as median (Interquartile Range between 25th and 75th percentile)Independent sample t-test was used to compare the means between two groups in case of parametric distribution Mann Whitney test was used to compare the medians between two non-parametric groups *p<0.05 was considered statistically significant.

Table 2: Characteristics of diabetic non-obese male subjects across different percentiles of leptin

LeptinPercentile†	$G_{< 5}$	G_{5-25}	G_{25-50}	G_{50-85}	G_{85-95}	$G_{>95}$	*p* value††
N (Male)	3	16	20	29	7	3	NA
% N with complications	100	100	80	10	17	0	NA
Age (Years)	45 (45,NA)	49 (46,52)	47 (41,51)	46 (42,49)	45 (42,50)	42 (42,NA)	0.593
BMI (kg/m^2)	19.1 (18.6,NA)	21.5 (19.9,21.9)	21.2 (20.4,22.2)	21.7 (20.6,22.5)	22.3 (20.4,22.5)	22.2 (20.2,NA)	0.087
Glucose (Fasting) mg/dL	161 (142,NA)	181 (132,274)	172 (121,196)	134 (109,163)	180 (106,209)	96 (95,NA)	0.062
Glucose (Postprandial) Lmg/dL	212 (199,NA)	296 (201,406)	277 (220,327)	206 (178,273)	294 (196,316)	216 (181,NA)	0.166
HbA_{1C}	8.7 (7.4,NA)	8.4 (7.6,9.7)	8.2 (7.8,8.6)	7.7 (7.1,8.1)	7.2 (7.1,8.0)	8.2 (8.0,NA)	0.010*
Urea mg/dL	23 (20,NA)	26 (21,32)	22 (19,25)	25 (21,30)	26 (22,34)	23 (18,NA)	0.352
Creatinine mg/dL	0.70 (0.60,NA)	0.75 (0.70,0.90)	0.75 (0.60,0.88)	0.70 (0.60,0.80)	0.70 (0.50,0.80)	0.80 (0.60,NA)	0.551
Cholesterol (Total) mg/dL	153 (137,NA)	177 (139,212)	178 (145,213)	189 (162,215)	170 (166,185)	228 (115,NA)	0.586
Triglycerides mg/dL	147 (91,NA)	122 (98,161)	149 (102,185)	122 (83,161)	131 (93,193)	164 (131,NA)	0.699

HDL mg/dL	42 (36,NA)	40 (35,43)	43 (37,47)	42 (40,47)	41 (39,44)	36 (28,NA)	0.219
UMCR mg/g	124 (64,NA)	84 (43,209)	54 (29,212)	27 (22,36)	30 (19,31)	28 (28,NA)	<0.001*
Insulin µIU/mL	5.75 (5.36,NA)	9.87 (9.33,10.56)	11.37 (10.99,11.90)	15.79 (14.79,17.04)	19.02 (18.76,19.18)	19.98 (19.54,NA)	<0.001*
Body at %	17.7 (16.5,NA)	20.6 (18.8,21.6)	19.7 (18.5,21.2)	20.4 (19.2,21.0)	20.1 (19.2,21.3)	20.1 (20.0,NA)	0.495
HOMA-IR	2.29 (1.88,NA)	3.99 (2.89,7.25)	4.64 (3.52,5.65)	4.77 (3.96,6.92)	8.34 (5.02,10.02)	4.88 (4.68,NA)	0.022*
HOMA-β	21.13 (12.91,NA)	30.14 (18.48,50.40)	40.05 (30.94,70.95)	82.30 (51.59,127.40)	57.72 (47.85,160.61)	224.43 (123.40,NA)	<0.001*
QUICKI	0.34 (0.32,NA)	0.31 (0.29,0.33)	0.31 (0.30,0.32)	0.30 (0.29,0.31)	0.28 (0.27,0.30)	0.30 (0.29,NA)	0.022*
Glucose Insulin Ratio	24.77 (23.29,NA)	17.64 (12.92,25.48)	14.01 (10.20,17.16)	8.27 (6.76,11.15)	9.32 (5.53,10.77)	4.76 (4.67,NA)	<0.001*

†Leptin values (ng/mL)

5th percentile: 6.04

25th percentile: 8.48

50th percentile (median): 9.65

85th percentile: 14.80

95th percentile: 15.22

Table 3: Characteristics of diabetic non-obese female subjects across different percentiles of leptin

Leptin Percentile†	$G_{<5}$	G_{5-25}	G_{25-50}	G_{50-85}	G_{85-95}	$G_{>95}$	*p* value††
N (Female)	3	14	18	26	6	3	NA
% N with complications	100	100	78	12	0	0	NA
Age (Years)	49 (36,NA)	50 (42,54)	44 (40,50)	46 (43,49)	42 (40,51)	42 (34,NA)	0.282
BMI (kg/m^2)	18.9 (18.5,NA)	21.4 (20.2,22.4)	21.5 (20.8,22.3)	21.6 (20.6,22.5)	22.5 (21.0,22.7)	21.5 (20.4,NA)	0.083
Glucose (Fasting) mg/dL	120 (104,NA)	132 (104,238)	177 (126,213)	136 (108,199)	125 (103,171)	130 (122,NA)	0.731
Glucose (Postprandial) mg/dL	184 (175,NA)	235 (157,386)	291 (215,368)	220 (186,302)	237 (165,283)	194 (158,NA)	0.154
HbA_{1C}	8.5 (7.2,NA)	8.1 (6.9,8.9)	8.3 (7.4,8.6)	7.9 (7.1,8.6)	7.1 (6.9,7.9)	6.8 (6.8,NA)	0.192
Urea mg/dL	21 (16,NA)	26 (20,30)	27 (20,32)	26 (19,31)	24 (22,29)	17 (17,NA)	0.726
Creatinine mg/dL	0.90 (0.60,NA)	0.80 (0.68,1.00)	0.75 (0.68,0.90)	0.80 (0.60,0.90)	0.70 (0.60,0.83)	0.60 (0.60,NA)	0.876
Cholesterol (Total) mg/dL	170 (152,NA)	194 (168,240)	186 (148,222)	183 (159,210)	156 (116,193)	158 (129,NA)	0.159
Triglycerides mg/dL	99 (95,NA)	149 (100,241)	104 (74,201)	109 (76,139)	100 (82,132)	144 (75,NA)	0.479
HDL mg/dL	40 (30,NA)	40 (37,41)	40 (38,45)	41 (39,45)	44 (40,48)	40 (40,40)	0.652

UMCR mg/g	263 (30,NA)	39 (33,174)	37 (30,104)	27 (21,31)	26 (18,29)	26 (16,NA)	0.001*
Insulin µIU/mL	9.26 (8.04,NA)	11.21 (10.43,11.60)	14.79 (12.84,18.48)	22.03 (20.23,22.84)	24.61 (24.10,24.90)	25.38 (25.25,NA)	<0.001*
Body at %	28.6 (25.1,NA)	31.5 (29.9,33.2)	30.5 (28.9,31.5)	30.9 (29.6,32.4)	31.27 (30.28,31.65)	28.74(28.7,NA)	0.093
HOMA-IR	2.70 (2.38,NA)	3.54 (2.95,6.86)	5.71 (4.98,8.13)	7.22 (5.71,10.92)	7.67 (6.30,10.16)	8.11 (7.65,NA)	0.001*
HOMA-β	58.50 (39.65,NA)	59.02 (23.05,97.15)	47.07 (37.21,104.70)	103.24 (56.38,167.03)	148.88(82.33,227.51)	135.65 (101.52,NA)	0.010*
QUICKI	0.33 (0.33,NA)	0.32 (0.29,0.33)	0.30 (0.28,0.30)	0.29 (0.27,0.30)	0.29 (0.28,0.29)	0.28 (0.29,NA)	0.001*
Glucose Insulin Ratio	12.08 (10.52,NA)	11.77 (9.12,21.32)	11.53 (7.07,13.59)	6.33(4.92,9.18)	5.07 (4.25,7.03)	5.15 (4.88,NA)	<0.001*

†Leptin values (ng/mL)

5th percentile: 7.66

25th percentile: 9.85

50th percentile (median): 15.25

85th percentile: 18.70

95th percentile: 19.89

All values are presented as median (interquartile range between 25th & 75th percentile) †† Comparison of non-parametric medians among multiple groups done by Kruskal Wallis Test *pvalue of <0.05 considered statistically significant

Table 4: Correlation of Leptin with insulin resistance indices in the presence or absence of long term diabetic complications among males and females

Male non-obese diabetic patients				
Leptin	**Without complications (N=39)**		**With complications (N=39)**	
	Correlation coefficient	***p* value**	**Correlation coefficient**	***p* value**
% Body Fat†	0.207	0.206	0.179	0.275
Fasting Insulin†	0.993	<0.001*	0.986	<0.001*
HOMA-IR††	0.421	0.008*	0.396	0.012*
HOMA β††	0.182	0.269	0.347	0.030*
QUICKI††	-0.421	0.008*	-0.396	0.012*
GI Ratio††	-0.319	0.048*	-0.455	0.004*
Female non-obese diabetic patients				
	Without complications (N=36)		**With complications (N=34)**	
Leptin	**Correlation coefficient**	***p* value**	**Correlation coefficient**	***p* value**
% Body Fat†	-0.040	0.819	0.187	0.290
Fasting Insulin†	0.987	<0.001*	0.995	<0.001*
HOMA-IR††	0.209	0.221	0.522	0.002*

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HOMA β††	0.156	0.363	0.213	0.227
QUICKI††	-0.209	0.221	-0.522	0.002*
GI Ratio††	-0.266	0.116	-0.423	0.013*

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† Pearson's correlation coefficient (r) used for correlating parametric distribution

††Spearman correlation coefficient (ρ) used for correlating non- parametric distribution *p<0.05 was considered statistically significant

Table 5: Multiple Linear regression analysis to test the effect of independent variables on leptin levels among non-obese diabetic male & female

Independent variables	Male non-obese diabetics			Female non-obese diabetics		
	B (95%CI)	β	*p* value	B (95%CI)	β	*p* value
Age	0.008 (-0.004 to 0.020)	0.015	0.205	-0.006(-0.019 to 0.006)	-0.008	0.300
Body fat %	-0.042(-0.082 to -0.001)	-0.025	0.043*	-0.020 (-0.059 to 0.019)	-0.008	0.313
Insulin(µIU/mL)	0.769(0.725 to 0.814)	0.970	<0.001*	0.789(0.760 to 0.817)	0.014	<0.001*
GI ratio	-0.018(-0.035 to 0.000)	-0.045	0.047*	-0.003(-0.024 to 0.018)	-0.004	0.792
HOMA-IR	-0.004(-0.059 to 0.051)	-0.003	0.885	-0.034(-0.070 to 0.003)	-0.029	0.070
HOMA- β	0.000(-0.001 to 0.002)	0.009	0.584	0.001(0.000 to 0.002)	0.018	0.093
	B – unstandardized coefficients β – standardized coefficients The dependent variable is Leptin F = 1966.78, p < 0.001 R^2 = 0.994 Adjusted R^2 = 0.994 *P<0.05 was considered statistically significant *Leptin (ng/mL) = 0.933 + [% body fat × (-0.042)] + [insulin(*µIU/mL*) × (0.769)] + [GI ratio × (-0.018)]*			B – unstandardized coefficients β – standardized coefficients The dependent variable is Leptin F = 4522.74, p < 0.001 R^2 = 0.998 Adjusted R^2 = 0.997 *P<0.05 was considered statistically significant *Leptin (ng/mL) = 1.056 + [insulin(*µIU/mL*) × (0.789)]*		

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Correlation of leptin with different indices of insulin resistance among cases and controls indicated a highly significant positive correlation for fasting insulin (r=0.993, p<0.001 for male controls; r=0.986, p<0.001 for male cases; r=0.987, p<0.001 for female controls; r=0.995, p<0.001 for female cases). Qualitative insulin sensitivity check index (QUICKI) as well as Glucose-Insulin ratio showed a significant negative correlation (QUICKI ρ= -0.421, p=0.008 for male controls; ρ= -0.396, p=0.012 for male cases; ρ= - 0.522, p=0.002only for female cases/ GI Ratio ρ= -0.319, p=0.048 for male controls; ρ= -0.455, p=0.004 for male cases; ρ= -0.423, p=0.013 only for female cases). Correspondingly, HOMA-IR and HOMA-β values were positively correlating with leptin. Percentage body fat does not seem to have a specific pattern of correlation for leptin for both cases and controls at 5% level among male and female. Results were indicated in table 4.Regression analysis was carried to assess the linear relationship of leptin with age, percentage body fat, fasting insulin levels, Glucose-Insulin ratio, HOMA-IR and HOMA-β. Results are summarized in table 5. All variables significantly predicted leptin levels for bothsexes (f = 1966.78, p< 0.001; adjusted R^2 = 0.994 for male; f = 4522.74, p< 0.001; adjusted R^2 = 0.998 for female). Only fasting insulin was found to add significantly to prediction of serum leptin statistically for both male [B = 0.769 (0.725 to 0.814);p<0.001) and female (B= 0.789 (0.760 to 0.817); p<0.001) diabetics. In case of male diabetics however, percentage body fat and Glucose-Insulin ratio also seems to add significantly to predict leptin levels [% body fat B= -0.042 (-0.082 to -0.001); p= 0.043/ GI ratio B= -0.018 (-0.035 to 0.000); p= 0.047]. Results of regression analysis indicate that for each one unit increase in fasting insulin, there is a corresponding increase in leptin level roughly by 0.769 ng/mL (with a maximum increase of 0.814 ng/mL)for male non obese diabetics and 0.789 ng/mL (with a maximum of 0.817 ng/mL) increase in female non obese diabetics. Interestingly, each 1% of decrease in percentage body fat and one unit decrease of GI ratio results in leptin level increase of 0.04 ng/mL and 0.02 ng/mL respectively, exclusively among male subjects.

Discussion:

The main aim of this study was to understand the changes of leptin levels with respect to insulin resistance indices and other possibly dependent demographic and biochemical characteristics among diabetic patients, in the presence or absence of any long term diabetic complications. Since the level of leptin is greatly influenced by age, adiposity and gender, we tried to rule out those confounders by selecting all the subjects between a narrow decadal age group of 35 to 55 years and with a normal body-mass index (18.5-22.9 kg/m^2). Gender related confounding was taken care by running separate statistics for both the sexes. Our results indicated that the prevalence of long term diabetic complications were maximumwhen there was a significant reduction in leptin level irrespective of sex of patient. Leptin levels were positively influenced by fasting insulin levels, insulin resistance index (HOMA-IR) and β cell functioning index (HOMA-β) irrespective of gender. They were negatively been influenced by Qualitative insulin sensitivity check index (QUICKI) and Glucose-Insulin ratio among both genders. Though the percentage of body fat was higher among female when compared to male gender, it showed a significant inverse relationship with leptin levels such that each 1% decrease of percentage body fat increases corresponding leptin values by 0.04 ng/mL, especially among male subjects.

The relationship between plasma leptin levels, fat mass, anthropometric factors, fasting insulin and insulin resistance indices have been studied extensively in normal as well as in diabetic adults. Shebi et al.[8] in their case control study on 80 individuals had found a

positive relationship between serum leptin and insulin resistance syndrome and that the leptin could play a major predictive role of insulin resistance syndrome. Also, they had a positive relationship between serum insulin and serum leptin (r = 0.51; $p<0.001$). Sayeed et al.[9] in their case control study on 50 non obese type 2 diabetic females and 50 age matched healthy controls had found a significant correlation of fasting insulin independent of BMI (r = 0.65; $p=0.007$) with overall reduction of leptin levels among diabetic subjects. Our results regarding the relationship between leptin and fasting insulin levels were consistent with the above findings.

Our findings also suggested a significant increase in the leptin levels as the index for insulin resistance (HOMA-IR) and index for β cell functioning (HOMA-β) increases. This could be explained by the fact that the relatively higher insulin levels among insulin resistant patients possibly stimulate the production of leptin from adipose tissue by down-regulation of hypothalamic leptin receptors or subsequent satiety response to leptin. Leptin receptors are present in hepatocytes and it was shown to modulate several insulin induced activities in these cells. Leptin antagonizes insulin signalling by decreasing insulin induced tyrosine phosphorylation of Insulin Receptor Substrate (IRS), increasing phosphoenolpyruvate carboxykinase and decreases glucokinase expression leading to increased gluconeogenesis and decreased glycolysis [10]. The hepatic effects of high leptin levels may in turn contribute to more insulin resistance thus setting in a vicious cycle. Also, leptin may act at different intracellular levels including transcription to membrane permeability to inhibit the synthesis and secretion of insulin [11].Functional leptin receptors are also present on insulin-secreting pancreatic β-cells [12], which could be a possible reason for insulin-lowering effect of leptin administration. Leptin also has a direct effect on insulin gene transcription in pancreatic β-cells with a reduction of preproinsulin m-RNA by 50% [13]. Furthermore, leptin affects the β-cell mass via changes in proliferation, apoptosis and cell growth [14].These could explain the gradual significant decrease of Qualitative insulin sensitivity check index (QUICKI) and Glucose-Insulin ratios, as the leptin levels increases in both male and female in our study.

Leptin is also able to modulate the various action of insulin like during glucose uptake and lipid synthesis in adipocytes and muscle cells, despite severe insulin deficiency, through its action in the brain [15]. Although it fully normalizes elevated plasma glucagon/ cortisol levels and reverses the increased hepatic expression of gluconeogenic enzymes, their effect on reduction of blood glucose level is only meagre [16]. The possible reason for gradual decrease in HbA1c levels in the presence of higher leptin,in our study, may possibly be due to the stricter glycemic control by treatment for type 2 diabetes mellitus rather thandue to relatively higher leptin.

Serum leptin was found to be significantly lower among patients with diabetic complications irrespective of the gender of the patient in our study. Also, the prevalence of diabetic complications were found to be more than 75% for both male and female diabetics when the leptin values were below 50^{th} percentile (which was 9.65 ng/mL for male and 15.25 ng/mL for female). Though leptin has an important role in the development of type 2 diabetes mellitus per se, its role regarding the development of long term diabetic complications are inconsistent. Koh et al. [17] in their study concluded that leptin therapy can cause thrombosis, by promoting platelet aggregation, leading to impaired endothelial function, immune function and foster inflammation and angiogenesis, all of them could possibly produce or worsen diabetic complications. Intravitreal leptin concentrations were found to be increased in individuals with proliferative diabetic retinopathy [18, 19]. Leptin has also been shown to stimulate ischemia-induced retinal neovascularization [20]. Leptin receptor deleted mice showed clinical evidence of autonomic neuropathy [21]. Elevated leptin concentrations, especially if infused exogenously was found to cause renal diseases in various experimental models [22]. *Lepr(+/+)C57BL/KsJ (db/db)* mice, which have a leptin receptor mutation, developed type 2 diabetes, characterized by comorbidities, commonly seen in humans including elevated systolic blood pressure, obesity, and hyperlipidemia [23].

It could be regarded that decreased leptin levels in the presence of diabetic complications in our study may primarily be due to drastic reduction in insulin secretion because of absolute loss of pancreatic beta cell functioning. This was evident by the fact that β-

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cell functioning index (HOMA-β) was found to be less than half, among diabetic patients, in the presence of complications compared to those without them [HOMA-β values of 34.15 (22.32,57.49) vs 88.59 (50.1,149.59), $p<0.001$ among male; 56.58 (35.32,97.15) vs 114.64 (58.92,169.5), $p=0.001$ among female]. Absolute insulin deficiency, in turn leads to decreased production of leptin from adipose tissue. The decrease in UMCR as the percentile leptin increases in table 2 and table 3 could have probably been due to the tighter glycemic control due to diabetic treatment (as evidenced by decreased HbA1c levels across percentiles) rather than the direct action of leptin in kidney functioning.

The close relationship of insulin and leptin and the presence of altered metabolism during diabetes could probably explain the absence of strong correlation between percentage body fat and leptin levels.

Conclusion:

We conclude that besides age, fat mass and gender, the main determinant of leptin levels among diabetic patients was insulin secretion and high degree of insulin resistance seem to contribute significantly to leptin level raise. Also, leptin level decreases drastically in the presence of long term diabetic complications though the decrease has possibly no influence with those developments.

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