Original Research Article

Serum leptin concentration in impaired glucose tolerance and recent onset type ii diabetes mellitus, relationship with anthropometry and lipid profile

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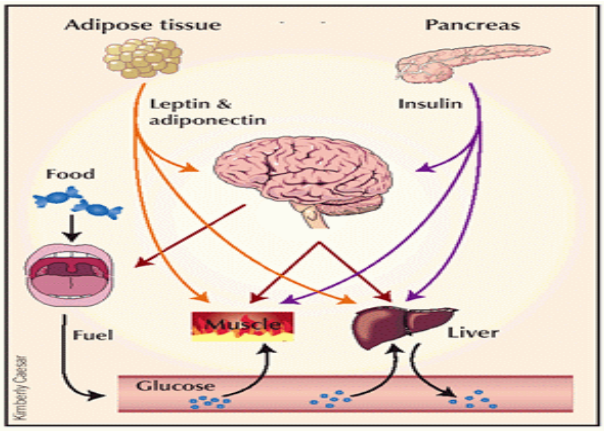
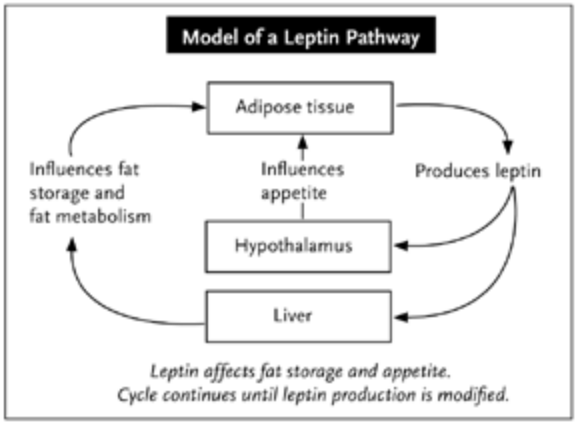
Abstract
Diabetes as well as pre-diabetes or impaired glucose tolerance increases the risk of cardiovascular disease by 2–3 times and increases by as much as 50% the risks of non-cardiovascular mortality associated with this condition. This study aim is to measure serum leptin levels in correlatation with anthropometry and lipid profile in pre- diabetes, non-diabetic and diabetic men and women. A cross-sectional study has been carried out in a total of 45 subjects for 20 subjects of pre-diabetes , 20 subjects of diabetic and 5 non- diabetic, south Indian rural 23 women and 22 men. Anthropometry was done for all the subjects with calibrated weighing machine, height scale etc. There is strong association between anthropometry and leptin resistance in both impaired and diabetic groups. In women both groups well correlated with total cholesterol and LDL. Increased serum Leptin levels/ leptin resistance was more evident in pre- diabetics when compared to non-diabetic and recent on set diabetes men and women.

Keywords: Diabetes, Cholesterol, Hdl, Ldl, Leptin.

Introduction
Diabetes as well as pre-diabetes or impaired glucose tolerance increases the risk of cardiovascular disease by 2–3 times and increases by as much as 50% the risks of non-cardiovascular mortality associated with this condition[1]. This high risk is not completely explained by the traditional risk factors[2,3]. Pre-diabetes is also associated with cardiovascular diseases (CVD)[4-6], but it is unclear if it is an independent risk factor, because it commonly co-exists with other cardiovascular risk factors present in the metabolic syndrome. Obesity and associated factors play important role in pre-diabetes in the Indian population. Serum leptin levels increase with body fat mass, as leptin resistance and not leptin deficiency. Role of this leptin resistance within India is not much studied in pre-diabetics. .In human beings, serum leptin concentration is directly proportional to body fat mass, but it is leptin resistance and not leptin deficiency *per se* which is regarded as a pathogenic mechanism in human obesity. Leptin concentrations vary widely among individuals with similar fat mass, indicating other possible factors for its determination[7-10]. Leptin may be a marker of risk of CHD, at least in males, and contributes to the CHD risk profile in subjects with insulin resistance[11-14]
This study aim is to measure serum leptin levels in correlatation with anthropometry and lipid profile in pre- diabetes, non-diabetic and diabetic men and women.





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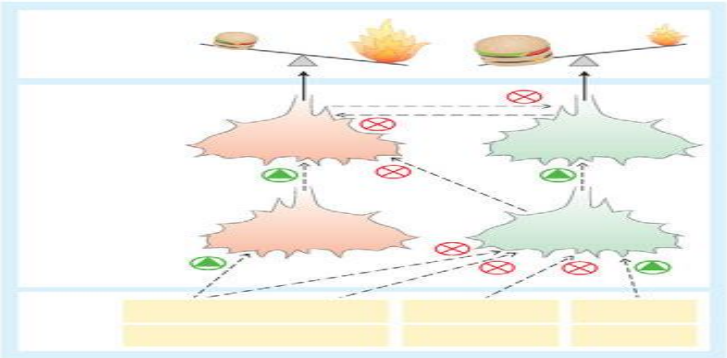


Fig No.1: Effectors PYY3-36 GLP-1 Leptin (adipose)Insulin(pancreas)PYY3-36 GLP1 (gut)Satiety signals Ghrelin(stomach)HungersignalAdipositysignals(gut)SatietysignalsGhrelin (stomach) HungersignalMuscleAdiposeLiverHypothalamusSecond-orderneuronsArcuate neurons Organs "Eat less, metabolize more" " Eat more, metabolize less" a-MSH NPY.

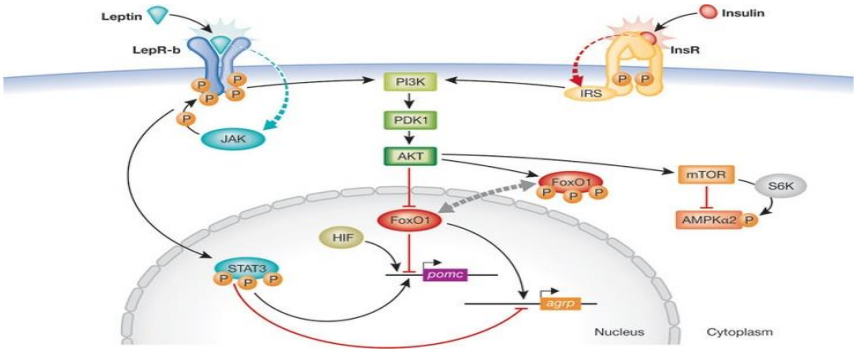


Fig No.2: A possible mechanism for cross talk between receptors for insulin and leptin. The insulin receptor has intrinsic Tyr kinase activity and the leptin receptor, when occupied by its ligand, is phosphorylated by a soluble Tyr kinase (JAK). One possible explanation for the observed interaction between leptin and insulin is that both may phosphorylate the same substrate—in the case shown here, insulin receptor substrate-2 (IRS-2). When phosphorylated, IRS-2 activates PI3K, which has downstream consequences that include inhibition of food intake. IRS-2 serves here as an integrator of the input from two receptors.

Materials and methods

A cross-sectional study has been carried out in a total of 45 subjects for 20 subjects of pre-diabetes , 20 subjects of diabetic and 5 non- diabetic, south Indian rural 23 women and 22 men. Anthropometry was done for all the subjects with calibrated weighing machine, height scale etc. Serum fasting leptin levels were measured by double sandwich ELISA method with Bio-rad fully automated ELISA reader. Lipid Profile was measured by Beck -man coulter fully automated bio-chemical analyser.

Fig No.3: ELISA READER

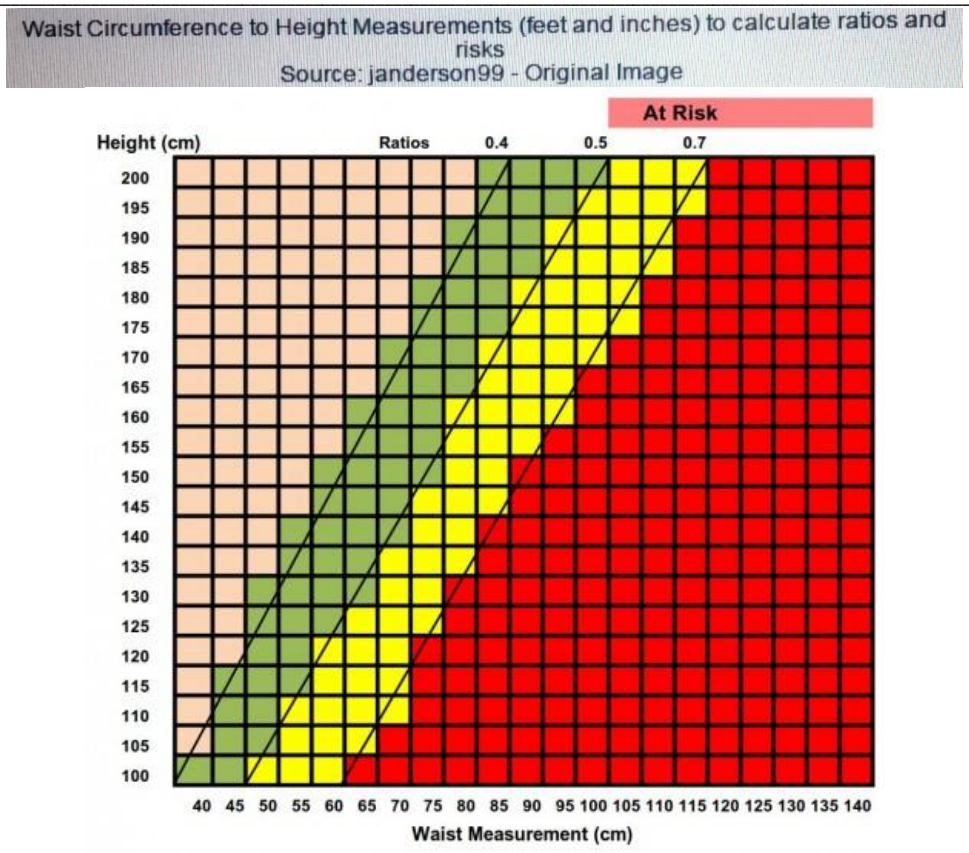


Fig NO4:Height measurements to caluclate ratios and risk

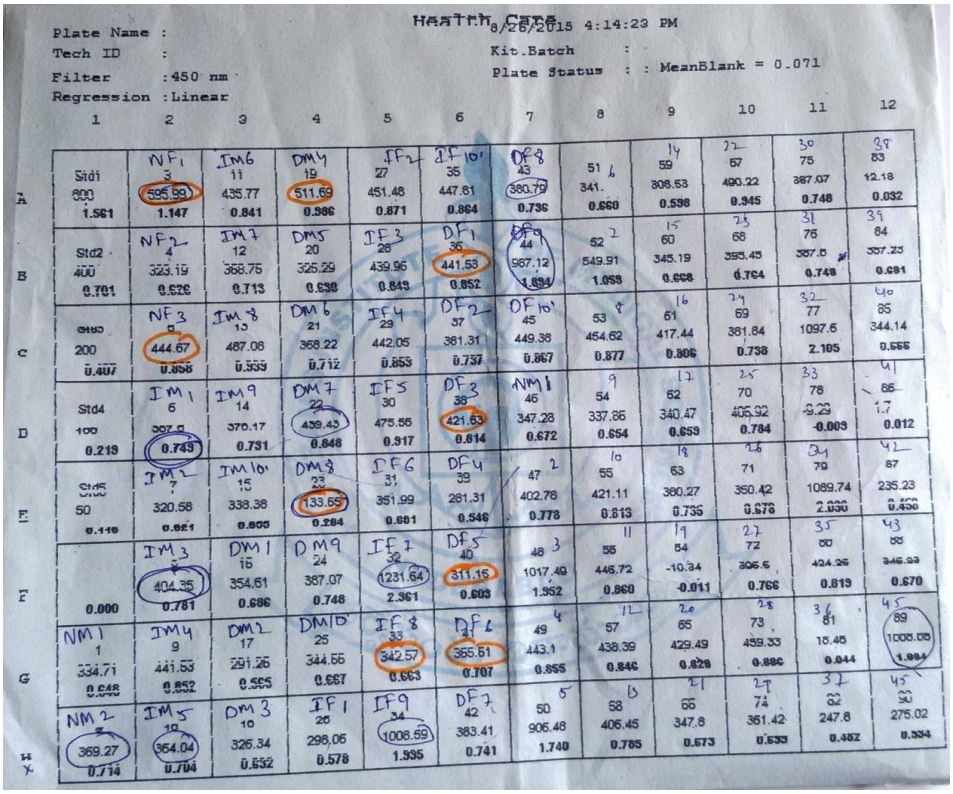
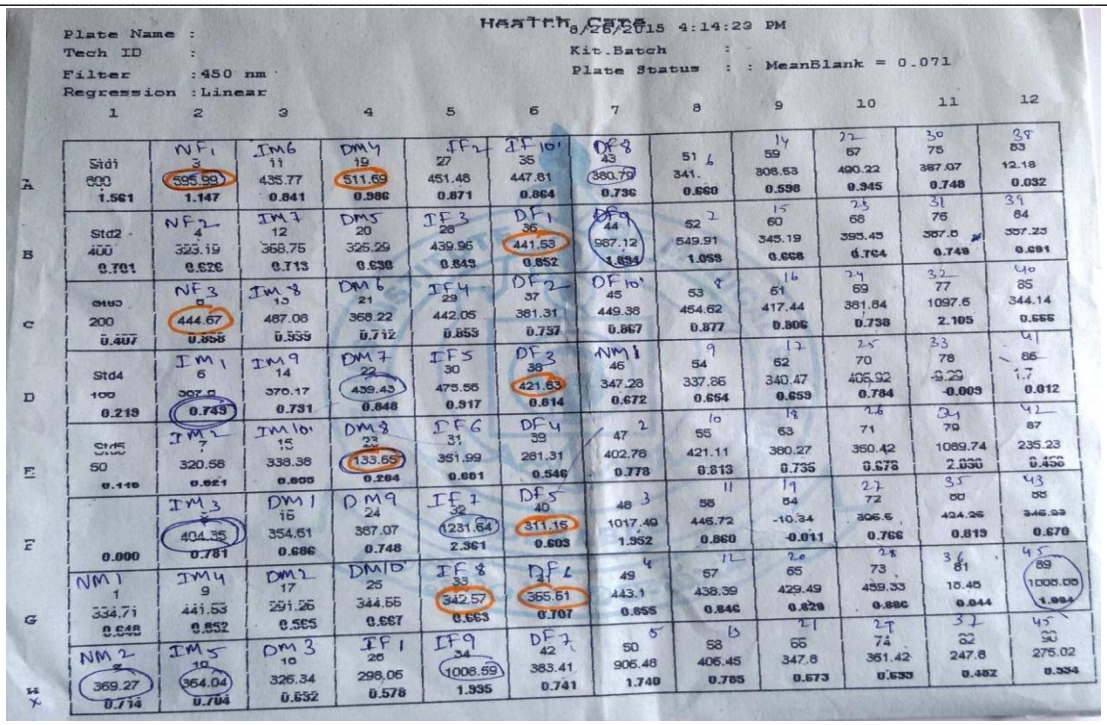


Fig NO.5 : caluclation of linear regression



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	1	2	3	4	5	6	7	8	9	10	11	12
A	Std1 800 1.561	NF1 3 595.99 1.147	IM6 11 435.77 0.841	DM4 19 511.69 0.986	IF2 27 451.48 0.871	IF10 35 447.81 0.864	DF8 43 380.79 0.736	51 6 341. 0.660	14 59 308.53 0.598	22 67 490.22 0.945	30 75 387.07 0.748	38 83 12.18 0.032
B	Std2 400 0.701	NF2 4 323.19 0.626	IM7 12 368.75 0.713	DM5 20 326.29 0.630	IF3 28 439.96 0.849	DF1 36 441.53 0.852	DF9 44 967.12 1.894	52 2 549.91 1.058	15 60 345.19 0.668	23 68 395.45 0.764	31 76 387.6 0.749	39 84 357.23 0.691
C	Std3 200 0.407	NF3 5 444.67 0.858	IM8 13 487.06 0.939	DM6 21 368.22 0.712	IF4 29 442.05 0.853	DF2 37 381.31 0.737	DF10 45 449.38 0.867	53 8 454.62 0.877	16 61 417.44 0.806	24 69 381.84 0.738	32 77 1097.6 2.105	40 85 344.14 0.666
D	Std4 100 0.219	IM1 6 397.0 0.765	IM9 14 376.17 0.731	DM7 22 439.43 0.848	IF5 30 475.56 0.917	DF3 38 421.63 0.814	NM1 46 347.28 0.672	9 54 337.86 0.654	12 62 340.47 0.659	25 70 405.92 0.784	33 78 -0.29 -0.003	41 86 1.7 0.012
E	Std5 50 0.110	IM2 7 320.58 0.621	IM10 15 338.38 0.655	DM8 23 133.69 0.264	IF6 31 351.99 0.681	DF4 39 281.31 0.546	47 2 402.78 0.778	10 55 421.11 0.813	18 63 380.27 0.735	26 71 350.42 0.678	34 79 1089.74 2.090	42 87 235.23 0.456
F	0.000	IM3 8 404.35 0.781	DM1 15 354.61 0.686	DM9 24 387.07 0.748	IF7 32 1231.64 2.361	DF5 40 311.15 0.603	48 3 1017.49 1.952	11 56 446.72 0.860	19 64 -10.34 -0.011	27 72 396.6 0.766	35 80 424.26 0.819	43 88 346.93 0.670
G	NM1 1 334.71 0.646	IM4 9 441.53 0.852	DM2 17 291.26 0.565	DM10 25 344.55 0.667	IF8 33 342.57 0.663	DF6 41 365.61 0.707	49 4 443.1 0.855	12 57 438.39 0.846	20 65 429.49 0.828	28 73 459.33 0.886	36 81 15.45 0.044	45 89 1000.00 1.931
H	NM2 2 369.27 0.714	IM5 10 364.04 0.704	DM3 16 326.34 0.632	IF1 26 298.06 0.578	IF9 34 1008.59 1.935	DF7 42 383.41 0.741	50 5 906.48 1.749	13 58 406.45 0.785	21 66 347.8 0.673	29 74 361.42 0.699	37 82 247.8 0.482	45 90 275.02 0.534

Fig NO.6: **caluclation of linear regression**

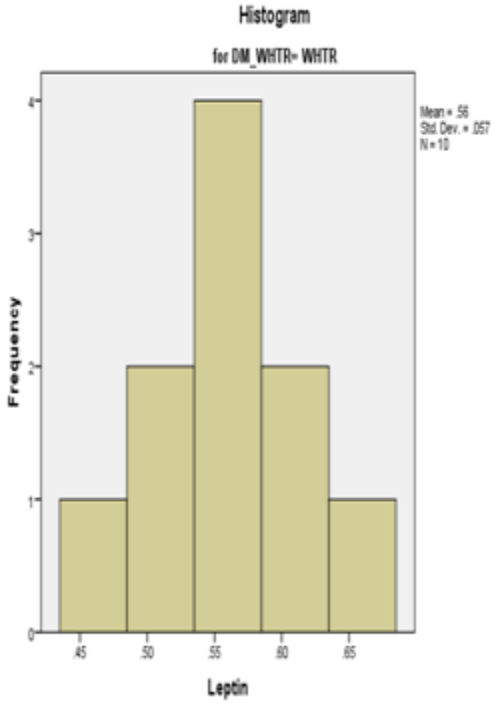
Figure NO.7: Obesity caused by defective leptin production. Both of these mice, which are the same age, have defects in the *OB gene*. *The* mouse on the right was injected daily with purified leptin and weighs 35 g. The mouse on the left got no leptin and consequently ate more food and was less active; it weighs 67 g.

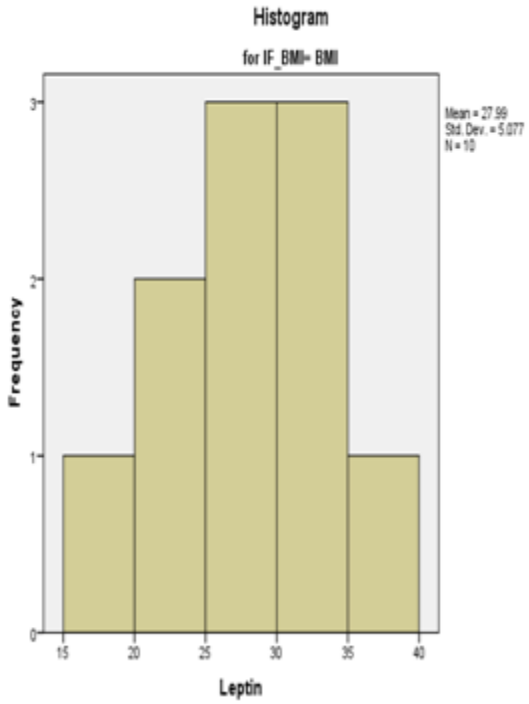
Results

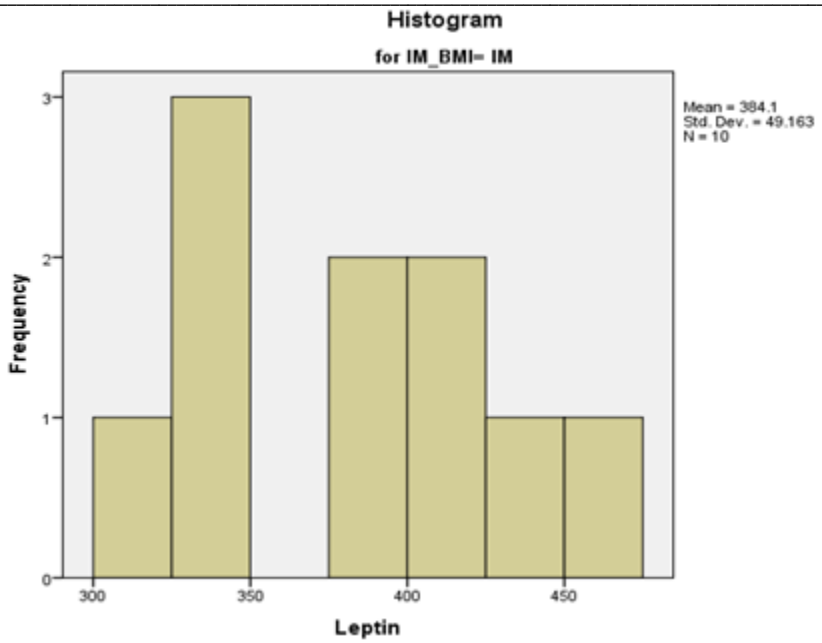
Data analyses were carried out by using the Statistical Package (SPSS 23.0). Kolmogorov-Smirnov was performed to test continuous variables for normality. Independent Student's t-test to compare means between groups of normally distributed data. P values <0.05 were considered significant. Leptin values increased with increased BMI(r-0.59), WHtR and HDL in both pre-diabetes and diabetes men and women (p value 0.000 to 0.009) and With increased total cholesterol and LDL , leptin values increased in both groups of women(p value 0.001 to 0.004). In diabetic men, impaired men and women leptin values showed strong association with triglycerides (p value 0.005 to 0.016).

Table No 1: BMI- Lipid Profile

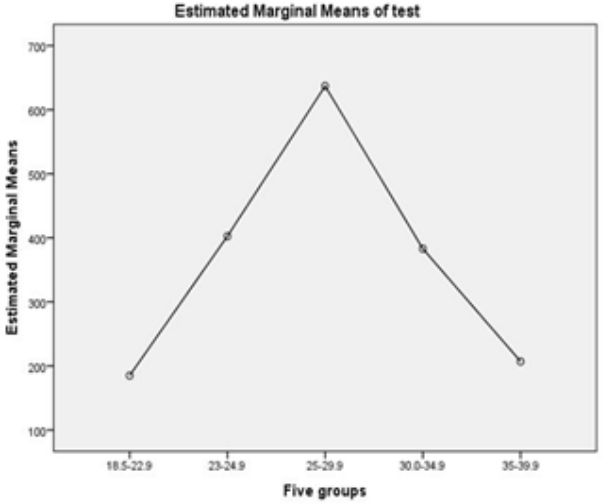
GR	AGE	WHR	FBS	WHtR	BMI	BODY MASS INDEX				LIPID PROFILE				LEPTIN
						BMI_UW <18.5	BMI_N 18.5-22.9	BMI_OW23-24.9	BMI_OB >24.9	TC	TG	LDL	HDL	
M(n=22)	46.72 ±10.58	0.91± 0.03	141.8(43.11)	0.54±0.048	25.36 ±2.55	0			25.36± 2.55	188.86±35.86	232.1 8±113.77	107.68±25.28	39.59 ±8.47	370.13±38.48
N(n=2	49.5 (10.5)	0.95± 0.05	90 (6)	0.55±0.055	21.25 ±0.35	0	21.25± 0.35			189±15	136± 33	125.5 ±11.5	36± 3	363.5 ±22.5
IGT=10	49.5 (11.9)	0.89± 0.03	111.4(5.72)	0.52± 0.054	25.9± 1.92	0			25.9±1.92	195.1±36.3	206.4 ±104.96	102.3 ±30.16	39.2 ± 9.4	384.1 ±40.48
DM=10	43.4 (8.28)	0.92± 0.02	182.6(39.6)	0.56± 0.04	25.65 ±3.04	0			25.65± 3.04	182.6±39.6	277.2 ±133.08	109.5 ±21.2	40.7 ± 8.3	357.5 ±37
FEMALE n=23	50.08 (6.62)	0.85± 0.06	131.17(37.90)	0.57± 0.06	26.98 ±3.77	0			26.98± 3.77	194.65±27.11	169.47±90.57	118.56±19.63	38.39±6.18	504.6 ±194.4
N n=3	41 (2.66)	0.78± 0.03	89.33(6.22)	0.51± 0.02	21.7± 2.13	0	21.7±2.13			164.33±21.11	85.33 ±11.11	102.66±8.22	44± 10.66	467.6 ± 68.8
IGT=n=10	51.4 (5.28)	0.85± 0.06	107.8(5.36)	0.587 ±0.039	27.99 ±3.83	0			27.99± 3.83	184.4±22.2	153.7 ±64.84	113.8 ±14.24	39.3 ±4.96	537± 228
DM n=10	51.5 (7.3)	0.86± 0.05	167.1(55.34)	0.62± 0.051	27.57 ±3.26	0			27.57± 3.26	214± 29.4	210.5 ± 61.44	128.1 ± 26.14	35.8 ± 7.04	483.4 ± 201.6



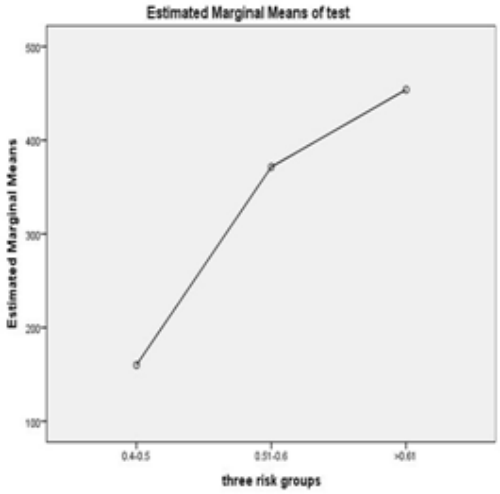




Leptin-BMI



Leptin- WHTR



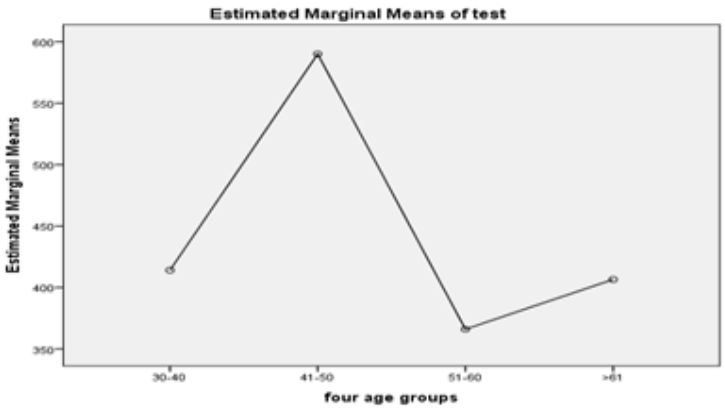


Table No.2: Case Processing Summary

	Df	Cases					
		Valid		Missing		Total	
		N	Percent	N	Percent	N	Percent
Leptin	DF	10	100.0%	0	0.0%	10	100.0%
LDL		10	100.0%	0	0.0%	10	100.0%

Table No 3: Tests of Normality

	DF	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Leptin	DF	.363	10	.001	.647	10	.000
LDL		.201	10	.200*	.939	10	.542

*. This is a lower bound of the true significance.
a. Lilliefors Significance Correction

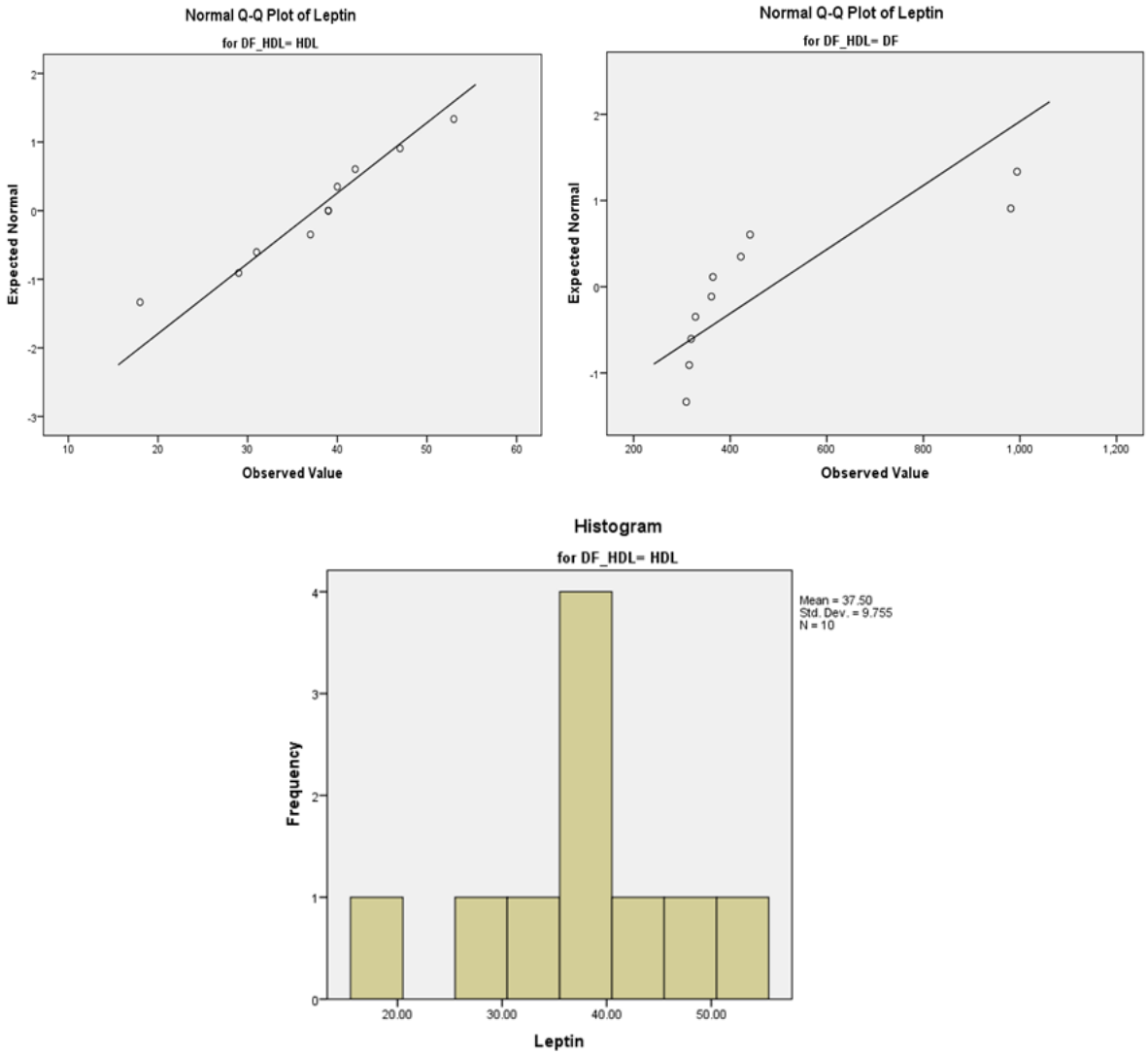


Figure No 8: Histograms of HDL & LDL

Discussion

South Asians, including Indian, Pakistanis, Sri Lankans and Bangladeshis serum leptin levels in a group of type 2diabetics residing in district Rawalpindi, Pakistan. The main findings of study are: type 2 diabetes is associated with marked reduction in serum leptin level in both men and women; serum leptin level is strongly associated with BMI in obese person- diabetics or non diabetics; in multiple regression analysis only BMI predicted serum leptin level. Serum leptin is found high in diabetics taking oral hypoglycemic, mean 37.8±19.1 ng/ml while it is low in diabetics taking insulin injections 29.3±24.2 ng/ml. This may be due to decreased insulin secretion in patients taking exogenous insulin. In some studies treatment of diabetes with sulfonylureas has been reported to increase serum leptin levels but not in other studies. In these studies, the effect

of sulfonylureas was mediated through changes in body weight or improved insulin secretion4 We observed a clear difference between BMI of both the groups. It is high in patients (n=30) receiving oral hypoglycaemics (mostly sulfonylureas) and low in patients (n=20) receiving insulin which is in accordance with above mentioned studies. This may be due to decreased insulin secretion in patients taking exogenous insulin. In some studies treatment of diabetes with sulfonylureas has been reported to increase serum leptin levels4 but not in other studies. In these studies, the effect of sulfonylureas was mediated through changes in bodyweight or improved insulin secretion. We observed a clear difference between BMI of both the groups. It is high in patients (n=30) receiving oral hypoglycaemics (mostly sulfonylureas) and low in patients (n=20) receiving insulin which is in accordance with above mentioned studies

An association between polymorphisms in the LEPR gene with glucose and insulin metabolism in overweight and obese women with IGT. This suggests that these genetic polymorphisms could affect the peripheral function of the LEPR in the regulation of insulin secretion and especially on insulin action. Fasting serum leptin levels ranged from 3.3–16.8 ng/mL (mean, 7.6 ng/dL). In contrast to glucose disposal, fasting serum leptin levels were highly correlated with sc, but not visceral, adipose tissue. As might be expected, serum leptin was not correlated with glucose disposal or plasma lipids. The correlation of leptin levels with BMI (r50.81, P,0.0001) reflects the high correlation of BMI. Data were presented according to gender, since it is already an established fact that leptin levels are significantly higher in women than in men. There are several possible explanations for the difference. One is that females have more adipose tissue than males, but a growing literature indicates that estrogen, especially at higher levels, will stimulate the production of leptin, whereas androgens will suppress the levels of leptin.

Conclusion

There is strong association between anthropometry and leptin resistance in both impaired and diabetic groups. In women both groups well correlated with total cholesterol and LDL. Increased serum Leptin levels/ leptin resistance was more evident in pre-diabetics when compared to non-diabetic and recent onset diabetes men and women.

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Conflict of Interest: Nil Source of support: Nil