Research Article

Leptin in non PCOS and PCOS women: a comparative study

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ABSTRACT

Background: Leptin, a hormone secreted by adipose tissues, controls body weight through regulation of appetite and thermogenesis. The present study was aimed to observe role of leptin in healthy and polycystic ovary syndrome (PCOS) women.

Methods: Correlation between serum leptin and anthropometric, endocrine and metabolic profile was studied in 30 apparently healthy women (control group) and 38 PCOS women (PCOS group). Each group was stratified based on body mass Index (BMI), as normal weight (BMI<23) and overweight/obese (BMI>23).

Results Leptin level was high in 30% control group and in 65.79% PCOS group. Mean leptin (ng/ml) in PCOS group was higher compared to control group (18±1.9 v/s 12±1.7, p<0.05). Mean leptin levels were higher in overweight/obese subgroup as compared to normal weight subgroup in both Control (p<0.05) and PCOS groups (p=<0.05). In control group, leptin showed positive correlation with waist circumference (WC) (r=-0.49, p<0.01) and negative correlation with Cholesterol: HDL ratio (p<0.05). In PCOS group, leptin showed positive correlation with BMI (r=0.377,p<0.05) and Triglyceride (r=0.34,<0.05) and negative correlation with Fasting Blood Glucose(FBG)(r=-0.33,p<0.05). In normal weight subgroup among control group (n=25), leptin showed positive correlation with LDL (r=0.49, p<0.05). In control overweight/obese subgroup (n=5), leptin showed positive correlation with Follicle Stimulating Hormone (FSH) (r=+1.0, p<0.05) and inverse correlation with testosterone(r=-1.0,p<0.05). In normal weight subgroup among PCOS group, leptin had a positive correlation with LDL: HDL ratio (r=0.488, p<0.05).

Conclusions: Hyperleptinemia is common in obesity. Leptin controls glycemic status in patients with IR. Correlation of leptin with FSH and testosterone is influenced by obesity and PCOS. Leptin regulation of lipid homeostasis is influenced by obesity or PCOS.

Keywords: Leptin, PCOS, Insulin

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the common endocrine disorders affecting five to ten percent women. This syndrome shows clinical features like oligo/amenorrhea due to anovulation, which may lead to infertility; hirsutism and acne due to hyperandrogenemia. Obesity, insulin resistance (IR) and dyslipidemia which may predispose patients to metabolic syndrome are common in PCOS.[1,2]

Leptin is a hormone secreted by adipocytes in proportion to total adipose tissue mass in body.[3] Its main role appears to control body weight through the regulation of appetite and thermogenesis.[4] Serum leptin levels vary depending upon the body fat stores; more the fat stores higher is the leptin level in blood. Increased leptin in circulation acts on hypothalamic leptin receptors to reduce appetite and food intake. If this feedback mechanism is working normally, obesity will not develop. In obese subjects, this feedback is faulty. They

either have structurally defective leptin or more commonly leptin resistance leading to hyperleptinemia. Thus leptin plays important role in long term energy regulation and lipid metabolism. Obese subjects as well as those with IR have hyperleptinemia hinting a possibility of coexisting mechanisms of IR and leptin resistance. Obesity and IR are common in PCOS; hence altered leptin levels/function in this syndrome is expected.

METHODS

The present study was conducted in 30 apparently healthy women (control group) and 38 newly diagnosed post pubertal PCOS women of reproductive age (PCOS group). The study was conducted a tertiary level Endocrinology and Research Centre. The women in control group were regularly menstruating and showed normal ultrasonography (USG).

The diagnosis of PCOS was fulfilled as per Rotterdam criteria[5]. Presence of at least two criteria from clinical, hormonal and abdominal USG category was considered diagnostic of PCOS.

Inclusion criteria for PCOS group

Women with complain of irregular menses or oligomenorrhea (absence of menses for 35-182 days) or amenorrhea (absence of menses for >182 days), signs or symptoms of hyperandrogenism, abdominal USG showing at least 12 follicles (two to nine mm in diameter) arranged peripherally around a dense core of ovarian stroma or scattered throughout an increased amount of stroma were enrolled in the study.

Exclusion criteria

Patients having any other major systemic illness including systemic inflammatory diseases, congenital adrenal hyperplasia, hyperprolactinaemia, acromegaly, functional hypothalamic amenorrhea were excluded from the study

The study was approved by the Institutional Ethics Committee. All the guidelines of Declaration of Helsinki were followed. Detailed menstrual history, marital status, parity were recorded in both the groups. The study subjects were clinically examined, subjected to pelvic ultrasonography and investigated for the endocrine and metabolic parameters.

Assay methods

Leptin was measures by enzyme-linked immunosorbent assay (ELISA) method. Hormonal levels were measured by sandwich immunoassay method. This included Luteinizing Hormone (LH) and Follicle stimulating Hormone (FSH). LH: FSH ratio was calculated. Serum Insulin was determined by in vitro quantitative determination of hormones was carried by electrochemiluminescence immunoassay method (Roche-Hitachi Cobas e 411). Blood glucose was measured on autoanalyser (Vital Scientific Microlab 300) using oxidase method. Lipid profile was estimated by quantitative enzymatic colorimetric method (GPO-PAP methodology) using Agappe diagnostic kits. This included Total Cholesterol (TC), Serum Triglyceride (Tg), Low-Density-Lipoprotein (LDL) High-Density-Lipoprotein (HDL).

Samples for fasting blood glucose (FBG) and fasting serum insulin levels were taken after overnight fasting. The venous blood sample was taken around 10 am. Homeostatic model assessment (HOMA) was calculated as marker of IR. HOMA value >1.9 was considered as presence of insulin resistance.

In the Control group the investigations were done from 6th to 12th day of menstrual cycle. In PCOS group, the investigations carried out were independent of day of menses because of irregular menses.

Anthropometric measurements were performed with participants in light clothing and with shoes removed. Height and weight were measured to the nearest 10th of a cm and kg, respectively. Waist Circumference (WC) and Hip Circumference (HC) were measured and Waist: Hip ratio (W:H) recorded by the same investigator using same calibrated machine over light clothing. Body mass Index (BMI) was calculated as weighing kilograms divided by the square of height in meters (kg/m^2).

Cut-off of BMI as Standard Consensus Statement for Indian population was considered[6], i.e.

Normal BMI: 18.0-22.9 kg/m^2; Overweight: 23.0-24.9 kg/m^2; Obesity: >25 kg/m^2 BMI $\geq$25.[9]

Statistical tests

Data was analyzed using statistical software (Graph pad prism version 5). Intergroup data was compared by unpaired 't' test. Fischers' test was used for qualitative data. Correlation was done by Spearman test. p value less than 0.05 was considered significant.

RESULTS

Serum leptin levels (ng/ml) ranged from 3.8 to 46 in control group, while in PCOS group it ranged from 3.2 to 58.00. The mean age of Control and PCOS groups was comparable. Thirty-four women (89.47%) in the PCOS group were infertile. (Data not shown)

Women in the control group had significantly less BMI than PCOS women (p<0.001) as well insulin (p<0.01) and HOMA (p<0.05). Women in PCOS group show dyslipidemic profile and significant for Triglycerides (p<0.05) and Cholesterol/HDL ratio (p<0.05) (Table 1).

Table 1: Comparison of anthropometric & biochemical parameters in control & PCOS groups.

	Control (n = 30)	PCOS (n = 38)	P Value
Weight (kg)	53.5±6.43	61.95±14.22	0.0037*
BMI	21.23±2.33	25± 4.9	0.0002*
Waist Circumference (inch)	38.27±21.3	51.12±24.26	<0.001*
LH/FSH	1.82 ± 1.79	1.7±0.84	0.756
FBG (mg %)	78.13 ± 9.58	82 ± 8.1	0.064
Insulin (µU/ml)	7.21 ±3.85	11±6.9	0.0158*
HOMA	1.47 ±0.91	2.2±0 1.7	0.035*
Testo (ng/ml)	27.94 ± 13.51	28± 19	0.980
Tg (mg%)	109.0 ±16.35	124 ± 31	0.021*
TC (mg%)	160.3±13.53	167±21	0.117
HDL (mg%)	41.93 ± 8.35	39 ± 4.7	0.0714
LDL (mg%)	89.7 ± 15.61	90 ±21	0.954
LDL/HDL	2.25 ±0.39	2.3±0.58	0.472
TC/HDL	3.97±0.55	4.3±0.64	0.017*

(mean±SD); BMI = Body Mass index, FBG= Fasting Blood Glucose, Testo = Testosterone, TC = Total Cholesterol, Tg = Triglyceride; * =p value significant

Low leptin levels (<5ng/ml) was observed in two women in PCOS group. Excluding these two women, the remaining (shown in shaded area; two by two table) was analyzed by Fishers' test. It shows that PCOS women are more likely to have high leptin levels (Odds ratio = 5.3) (Table 2).

Women in control group have lower leptin levels than in PCOS (p<0.05). In control group, overweight/obese subjects had higher leptin levels than normal weight (p<0.05) (Table 3).

Table 2: Percentage of women showing low, normal and high leptin levels.

	Low Leptin (<3.7 ng/ml)	Normal Leptin (3.7 – 11.1ng/ml)	High Leptin (>11.1 ng/ml)
Control (30)	Nil	70% (21)	30% (9)
PCOS (38)	6.81% (2)	28.95% (11)	65.79% (25)
p value		0.0027** Fischer's	

Figures in parenthesis =number of subjects, **- value – highly significant

Table 3: Percentage of women showing low, normal and high leptin levels.

Group	Mean ±SE	P value PCOS overall	Control obese	PCOS normal wt.	PCOS obese
Control overall (30)	12± 1.7	0.0402*	-	-	-
PCOS overall (38)	18 ± 1.9	-	-	-	-
Control normal wt. (25)	10.20±1.37	-	0.0227*	0.978	-
Control overweight /obes (5)	21.76±6.63	-	-	-	0.876
PCOS normal wt. (16)	13.00±2.5	-	-	-	0.0737
PCOS overweight/obese (22)	21±2.5	-	-	-	-

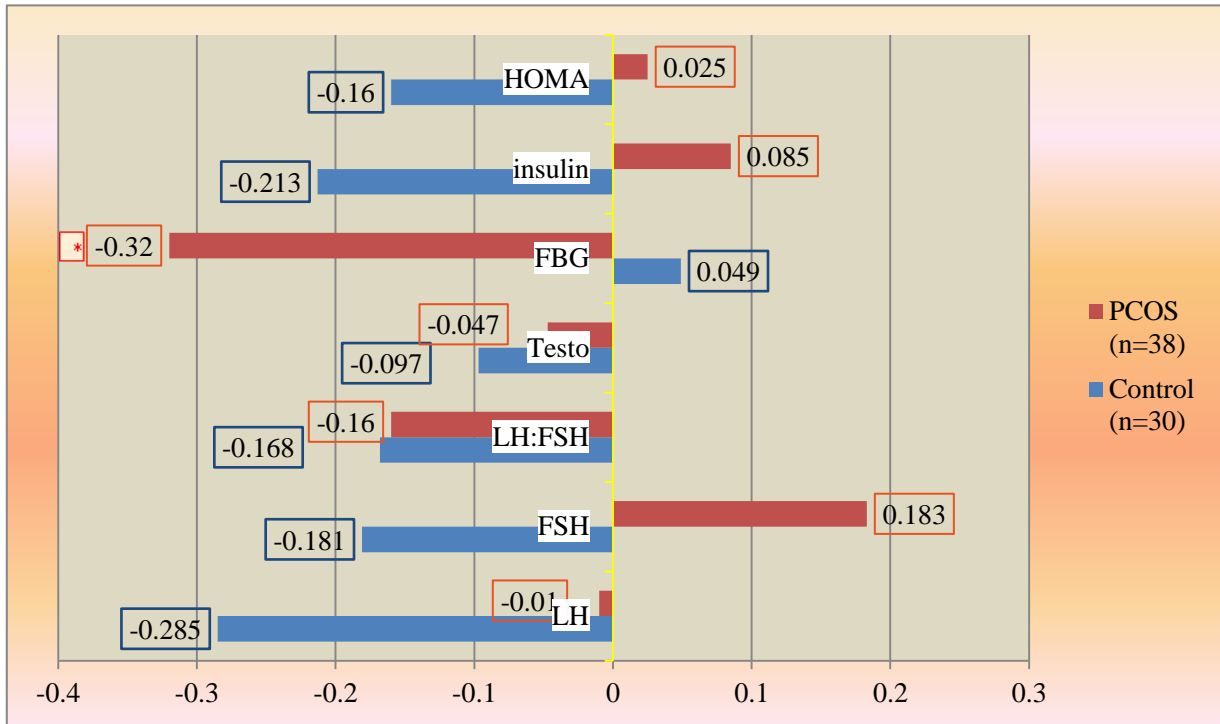Figures in parenthesis =number of subjects, *- p value significant

Figure 1: Correlation between leptin and hormones, glycemic parameters, in control and PCOS groups.

Testo = Testosterone; * p value >0.05 (significant)
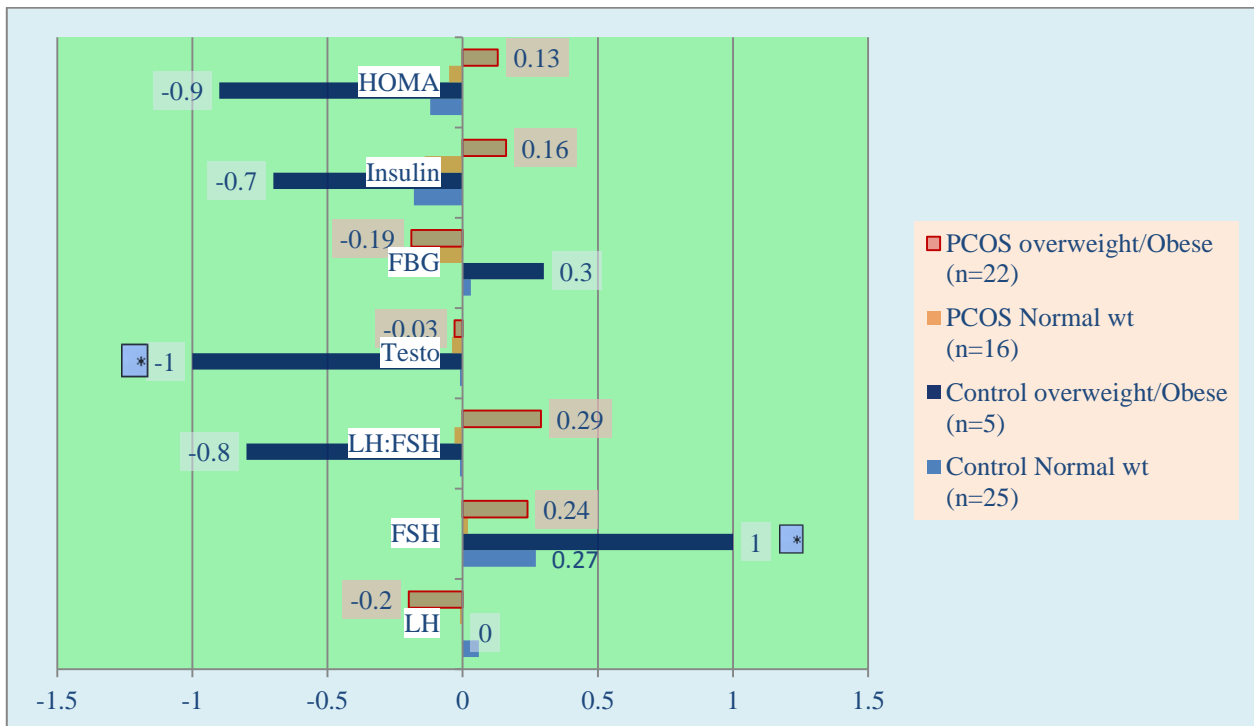

Figure 2: Correlation of leptin with hormones and glycemic parameters in control and PCOS subgroups.

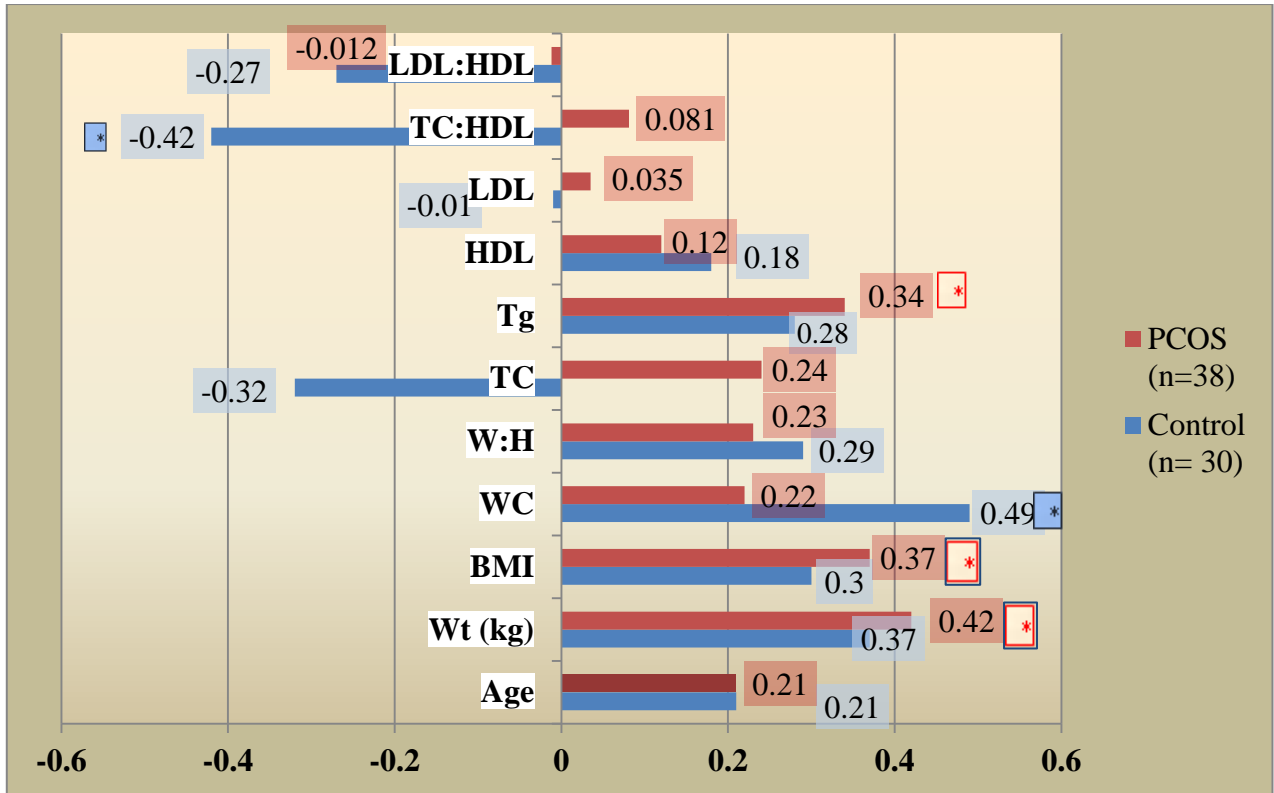Testo = Testosterone; * p value >0.05 (significant)

Figure 3: Correlation of leptin with anthropometric and lipid profile in control and PCOS groups.

Wt = Weight, WC = Waist Circumference, W:H= Waist: Hip ratio, TC = Total Cholesterol, Tg = Triglyceride; * =p value significant

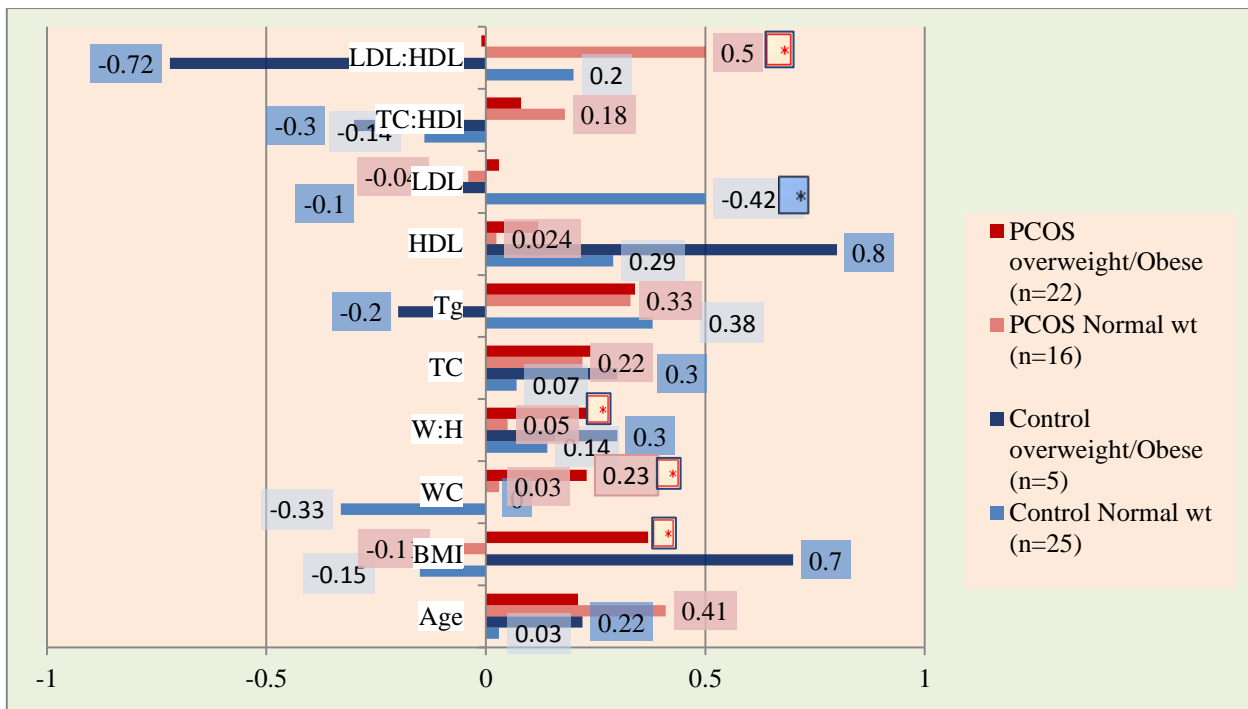Leptin and age did not show significant correlation in both the groups

Figure 4: Correlation between leptin and age, BMI, W: H, lipids in control group and PCOS subgroups.

WC = Waist Circumference, W:H= Waist: Hip ratio, TC = Total Cholesterol, Tg = Triglyceride; * =p value significant

DISCUSSION

In the present study, control group and PCOS group differed significantly with respect to BMI, glycemic status and some of the lipid profile parameters (Table1). Significantly higher BMI, fasting insulin and HOMA in PCOS group indicates presence of IR, while higher Tg indicates presence of deranged lipid metabolism. These results are in accordance to the results of the previous studies. Obesity is common in PCOS and these women are more prone to develop IR and subsequently type 2 diabetes mellitus.[1]

The results of the present study show that the percentage of women showing higher leptin levels was more in the PCOS group as compared to the control group (Table 2). Hyperleptinemia in 29% of both obese and nonobese PCOS women was reported in the earlier studies.[7] The mean leptin level in the PCOS group was significantly higher in the PCOS group as compared to the control group (Table 3). Laughlin et al reported serum leptin levels in PCOS women did not differ from BMI matched normally cycling women.[8] The divergent result of the present study may be because 57.89% of PCOS women were either overweight /obese (Table 2).

On stratifying the data based on BMI, both control and PCOS groups showed higher leptin levels in overweight/obese women as compared to normal weight women of the respective groups (Table 3). The difference was not significant. Hence raised leptin levels in both the control and PCOS groups may be attributed to obesity.[9] Association between obesity and raised leptin levels has been reported previously and is well known. Serum leptin concentrations correlate with the percentage of body fat, suggesting that most obese persons are insensitive to endogenous leptin production.[10]

Two PCOS women showed less than normal serum leptin levels. These two women may represent a subgroup of leptin deficient PCOS (Table 2). Though it is reported that decreased leptin production relative to the amount of body fat is seen in about 5% of obese humans, none of the women with hypoleptinemia was obese (one normal weight and other overweight).[11] It is proposed that some PCOS women may be leptin deficient, thus are subject to pathophysiological changes due to hypoleptinemia. Further studies in this direction are welcome.

On analyzing the correlation of leptin with glycemic parameters it was observed that in control group, insignificant negative correlation existed between leptin and fasting insulin as well as HOMA (Figure 1). Correlation between leptin and insulin is well documented.[12] In control group, insignificant positive correlation was seen between leptin and FBG (Figure 1). This is physiological as blood glucose influences appetite and leptin is appetite suppressant. The lack of significant level can further be explained as in control group, leptin is not related to FBG significantly because physiological glycemic homeostasis is maintained by normal insulin levels and or function. In contrast, in PCOS group, leptin and FBG were significantly (p<0.05) and inversely related. The physiological inverse relation between leptin and insulin, HOMA is disturbed. The correlation between leptin and fasting insulin as well as HOMA was insignificantly positive (Figure 1). This may be because of presence of leptin resistance with respect to insulin secretion. At the same time, leptin may be functioning through other noninsulin pathway to maintain blood glucose level in presence of IR. This is further substantiated by the fact that these women showed weaker correlation between FBG and fasting insulin (r = 0.040, p=0.809, data not shown) as compared to that between FBG and leptin. Even on stratifying the group based on BMI, the correlation of leptin with FBG and insulin was insignificant in PCOS subgroup (Figure 2) Together these results indicate firstly, leptin has no or minimal role in maintaining fasting blood glucose level and or carbohydrate metabolism if insulin homeostasis is intact. And secondly, in PCOS or obesity, the IR state reinforces leptin to control blood glucose levels or carbohydrate metabolism through insulin independent pathways. In this sense leptin may be acting as a second in command to maintain carbohydrate metabolism.

Leptin has important effects to stimulate Luteinizing Hormone Release Hormone (LHRH) from hypothalamus and also to stimulate FSH and LH release from the pituitary in experimental animals.[13] Leptin has been proposed to serve as a signal relating nutritional status to hypothalamic regulators of reproductive function.[14] Leptin has been shown to correlate inversely with LH levels, independent of body weight and IR.[15] In the present study correlation analysis between leptin and LH, FSH showed lack of significant correlation in control and PCOS groups (Figure 1). This result is similar to the observation reported by Pehlivanov B.[16]

A positive correlation of leptin with FSH, and inverse correlation with testosterone in obese PCOS in subgroup alone is interesting. FSH and testosterone were correlated inversely with similar level of significance (r = -1, p=0.0167 data not shown).This subgroup had less number of subjects (n=5) and leptin levels are on higher side in the obese. In PCOS, in spite of higher levels, leptin has no relation with FSH and testosterone. Obese women in control and PCOS groups showed comparable leptin levels (Table 2). In PCOS, the relay signal of Leptin with gonadotrophin function may be faulty with/without leptin resistance. The comparison of correlation of leptin with gonadotropins in control and PCOS group should be considered on the fact the investigation was done between 6- 12 day of menstrual cycle in the former group which was not possible in the latter group.

FSH secretion is reduced in PCOS. Leptin plays role in reproductive function. Leptin is involved in gonadal function and fertility. The relatively weaker association

between leptin and FSH can be explained by presence of leptin resistance in PCOS women.

With testosterone, leptin had no correlation in the control group and a weak inverse correlation in the PCOS group. In normal weight control women the nonexistence of correlation persisted while the obese control women showed a highly significant inverse correlation. Normal weight PCOS women showed result similar to normal weight control women while obese PCOS subgroup showed insignificant inverse correlation. Segal et al reported higher leptin levels in females than in males after correction with body fat mass; suggesting that androgens could have a suppressive effect on leptin levels.[19] It is also possible that leptin suppresses testosterone levels.

The significant positive correlation between leptin and WC in the control group is in accordance to the previous reports (Figure 3). Failure to reach the level of significant positive correlation of leptin with BMI in this group may be because of less number of obese women in this group. Sorensen et al reported that serum leptin levels are highly correlated with measures of body adiposity including BMI. BMI and WC provide simple yet sensitive methods for the estimation of total and central adiposity in groups of adult women.[20] Correlation of leptin with BMI was highly significant in PCOS women. Our reports are similar to Mantzoros et al who found positive correlation between leptin and BMI in women with PCOS (r = 0.70; p<0.001) and weight- and age-matched Controls (r = 0.59; p < 0.05).[21] Unlike Control normal weight subgroup, PCOS normal weight subgroup lacked significant correlation between leptin and BMI (Figure 4). The failure to reach level of significance may be because of less normal weight subjects in PCOS group. Leptin in PCOS obese subgroup had a significant correlation with BMI, body weight and WC.

The significant inverse correlation between leptin and TC: HDL in control group reflects favorable role of leptin in lipid metabolism (Figure 3). The significant positive correlation with LDL and LDL: HDL alone in normal weight subgroups in both control and PCOS women respectively. Fig 4 indicates BMI based degree of difference influence of leptin on lipid homeostasis. PCOS women had a significant positive correlation between leptin and Tg. Leptin indicates amount of Tg stored as adipose tissue which in turn is reflected by serum Tg levels. Leptin is an appetite suppressant and influences lipid metabolism favorably. Previous studies[22] suggest that leptin is associated with factors regulating fuel homeostasis and its hormonal control in man. The authors concluded that serum leptin concentrations correlate directly with triglyceride. First, under conditions of steady-state energy balance, leptin is a static index of the amount of Tg stored in adipose tissue.[4] Relation of serum leptin and lipids is not yet fully explored. Haluzik et al in a comparative study between healthy and untreated hyperlipedimic subjects concluded that leptin reflects body fat content and have no significant relation with lipids or lipoproteins.[23] Nonetheless the present results indicate that the manipulation of lipid homeostasis by leptin may be influenced BMI and disease conditions like PCOS.

CONCLUSION

Hyperleptinemia is common in obesity. Hyperleptinemia is noted in PCOS and is because of obesity. Some patients of PCOS women present with hypoleptinemia. In PCOS patients with obesity and concomitant IR, Leptin takes Control of glycemic status via insulin independent mechanism. PCOS women may have faulty leptin gonadotrophin relay with or without leptin resistance, which is reflected by decreased FSH secretions. Correlation of leptin with FSH and testosterone is influenced strongly by obesity and PCOS. Leptin regulation of lipid homeostasis may be influenced by body weight or PCOS.

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