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Original Research Article

Hyperleptinemia is correlated with Metabolic Syndrome Risk Factors in Women with Polycystic Ovarian Syndrome

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ABSTRACT:
Polycystic ovarian syndrome (PCOS) as a common cause of anovulatory, is associated with glucose metabolic abnormality, hyperinsulinemia and obesity, features which are related to leptin and its receptors. However, there is still debate over the link between leptin and endocrine and metabolic factors in PCOS. The present study aim to compare both obese/overweight and normal/underweight PCOS and healthy women regarding anthropometric and endocrine parameters. Also, estimate inter-relationship of serum leptin levels with anthropometric and endocrine parameters. This is a case-control study including 150 PCOS women and 100 control subjects. Anthropometric indices were measured for each subject. Lipid profile, fasting glucose, insulin, gonadotropin hormones, testosterone and serum leptin were estimated. Insulin resistance through HOMA-IR model and insulin sensitivity through (QUICKI) were calculated. Student's t-test and two-way ANOVA were used for analyzing the differences between groups. Pearson (r) correlation coefficient was used to assess the correlation between serum leptin and the independent variables. Waist circumference was increased in normal/underweight PCOS group compared to normal/underweight control group. Fasting glucose and insulin, HOMA-IR, QUICKI and serum leptin levels were significantly increased in obese/overweight PCOS group compared to obese/overweight control group. Strong significant correlation was observed between serum leptin levels and increased BMI, LDL, fasting glucose, fasting insulin, HOMA-IR and hypotension. Serum leptin levels showed strong correlation with metabolic syndrome risk factors especially glucose metabolic abnormality in PCOS women compared to control.

Keywords: *Polycystic Ovary Syndrome; LeptinSerum, Metabolic Syndrome, Anthropometric Indices, Endocrine Parameters*

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INTRODUCTION

PCOS (polycystic ovarian syndrome) is an endocrine disorder that affects women of reproductive age. According to the ethnicity and diagnostic criteria applied, PCOS prevalence varies between 5 to 26% (Aziz et al., 2006; Sirmans and Pate, 2014).

Obesity, dyslipidemia, insulin resistance, type 2diabetes, and hyperinsulinemia are all possible metabolic consequences of PCOS. These risk factors contributed to the development of metabolic syndrome (MetS) and, as a result, increased the risk of cardiovascular disease (CVD) in PCOS women (Mani et al., 2013).

Leptin hormone secreted by adipose tissue and encoded by the *'ob'* gene influence fertility, angiogenesis, hematopoiesis, and immune functions (Snoussi et al., 2006). Obesity may affect ovarian functions in PCOS women due to increased intra-follicular amounts of leptin, and it may induce comparative resistance to gonadotropins (Pinilla et al., 1999). Studies have reported that about 30–75% of the PCOS subjects are obese, and also 50–60% has central adiposity regardless of their body mass index (BMI) (Horejsi et al., 2004; Ehrmann et al., 2006). Leptin concentrations fluctuate during the menstrual cycle, indicating that leptin and the hypothalamic-pituitary-ovarian (HPO) axis may have a feedback interaction in adult women (Goldsammler et al., 2018). The presence of leptin receptors in granulosa cells, which collaborates with glucocorticoids to enhance steroidogenesis, suggests that leptin regulates ovarian folliculogenesis directly (Sir-Petermann et al., 1999).

A study has reported that leptin levels increased in obese and non-obese subjects with PCOS compared to control (Yildizhan et al., 2011). However, the association studies between leptin levels and PCOS have demonstrated contradictory results (Mendonça et al., 2004; Glintborg and Andersen, 2010). Insulin resistance and hyperinsulinemia may also be factors which influence serum leptin levels. Insulin has been shown to directly induce leptin mRNA in adipocytes in vitro, implying that insulin can promote leptin secretion (Nasrat et al., 2016). Higher leptin levels can be associated with metabolic disorder, insulin resistance, infertility, and even CVD in PCOS, although there is a high degree of variation in the findings; that could be due to the etiology and progression of PCOS (Zheng et al., 2017).

This study aims to a) compare both obese/overweight and normal/underweight PCOS and healthy women using anthropometric and endocrine parameters and b) estimate the inter-relationship of serum leptin levels with anthropometric and endocrine parameters.

MATERIALS AND METHODS

A total of 250 individuals, including 150 PCOS patients and 100 controls, were selected from governmental and private hospitals from Mysore, Karnataka, India.

This case-control study was approved by the Institutional Human Ethical Committee (IHEC), University of Mysore, Manasagangothri, Mysore (IHEC-UOM No. 151/PhD/2017-18), following the Helsinki Declaration (as revised in 2013), and written consent was obtained from all the subjects.

In the present study, the following inclusion criteria were used: PCOS women in the reproductive age range of 18-45 years were diagnosed by Rotterdam criteria that require the presence of at least two criteria out of the following three (ESHRE and ASRM-Sponsored PCOS Consensus Workshop Group, 2004):

- Oligo- and/or anovulation – whether or amenorrhea was present.
- Clinical and/or biochemical signs of hyperandrogenemia
- Polycystic ovaries (PCO) on ultrasound characterized by ≥ 12 follicles with the measurement of 2–9 mm in diameter, or ovarian volume should be more than 10 ml in at least one ovary.

The diseases with similar clinical features to PCOS include Cushing's syndrome, congenital adrenal hyperplasia, adrenal 21-hydroxylase deficiency, thyroid dysfunction, androgen-secreting tumors, hyperprolactinemia were excluded.

The control group was selected from healthy volunteer women aged 18-45 years with regular ovulating cycles besides normal ultrasound presence of the ovaries and without any history of dyslipidemia, thyroid disorder, insulin resistance, diabetes, hypertension, hyperandrogenism. Investigations were conducted by collecting each person's health history and documents, filling the genetic registry, and confirming the information by clinical and hormonal analysis.

General information (age, marital status), reproductive information (menstruation, fertility), clinical features of PCOS, drug history were collected from each patient.

Weight, height, hip, and waist were measured precisely. All measurements of the subjects were taken while standing, with feet together, the abdomen was relaxed, and the arms at the sides. BMI (weight (kg)/height^2 (m)), waist to hip ratio (WHR), waist to height ratio (WHtR) were calculated. Obese/overweight and normal/underweight groups were defined based on BMI ≥ 25 and BMI <25, respectively (WHO, 2000). Systolic and diastolic blood pressures (SBP and DBP) were measured with the subjects in the sitting position on the right hand.

During the 2nd to the fifth day of the menstrual cycle or any day in amenorrheic patients, 5 ml peripheral venous blood samples were collected from each subject after a 10-12 h overnight fast.

Lipid profile including cholesterol, triglycerides (Meril diagnostics, India), and high-density lipoprotein (HDL) (Erba Mannheim, Germany) were estimated by enzymatic assay. Low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were calculated by the Friedewald equation (Friedewald et al., 1972; Cleeman et al., 2001). Fasting glucose was measured by the Glucose Oxidase-Peroxidase method (ARKRAY Inc., Japan). Fasting insulin hormone was estimated by the ELISA method (CALBIOTECH, USA).

Insulin resistance was calculated by homeostasis model assessment (HOMA-IR) index= (fasting plasma insulin [in microunits per milliliter] × fasting plasma glucose [in milligrams per deciliter])/405 (Legro et al., 2004). The insulin sensitivity was calculated by the quantitative insulin sensitivity check index (QUICKI) =1/log fasting insulin (in microunits per milliliter) + log fasting glucose (in milligrams per deciliter) (Katz et al., 2000).

Gonadotropin hormones, including luteinizing hormone (LH) and follicle-stimulating hormone (FSH), were estimated by the ELISA method (Meril diagnostics, India). Testosterone levels were estimated with an enzyme-linked fluorescent immunoassay method using a mini VIDAS instrument (the Compact Automated Immunoanalyzer) and BioMérieux testosterone kit (Biomerieux, France). Serum leptin hormone was estimated by ELISA method based on sandwich technique (DRG, USA).

Statistical analysis

Results were presented as mean ± standard deviation (SD). The Shapiro–Wilks test was applied to determine the normality of continuous variable data distribution. Student's t-test and two-way analysis of variance (two-way ANOVA) were used for analyzing the differences between groups. Homogeneity of variance was examined using Leven's test. Pearson (r) correlation coefficient was used to assess the correlation between serum leptin and the independent variables. Statistical significance was defined as a $P < 0.05$. Statistical analysis was performed using the Statistical Package for the Social Sciences software SPSS, version 23.0; IBM (IBM Corp. Armonk, NY, USA).

RESULTS

A total of 250 participants were fulfilled for the study. The PCOS and control samples' mean age was 25.78 ± 5.00 and 24.79 ± 4.66 years. The BMI distribution was 47% normal/underweight and 53% obese/overweight in PCOS and 54%, 46% in controls, respectively, indicating that the frequency of obese/overweight women with PCOS was higher than controls. Anthropometric indices defining central obesity, such as WC and WHtR, were significantly elevated in the PCOS group compared to the control group.

Anthropometric and endocrine characteristics are presented in Table 1; BMI,

WC, WHR, WHtR, DBP, fasting glucose, fasting insulin, cholesterol, triglycerides, HDL, LDL, serum leptin, HOMA-IR, QUICKI, LH, LH/FSH, and testosterone were shown to be significantly different between PCOS and the control group.

Table 1: Comparison of anthropometric and endocrine characteristic between PCOS and controls

Analyses	PCOS (n = 150)	Control (n = 100)	*P-value*
Age (year)	25.78 ± 5.00	24.79 ± 4.66	0.114
BMI (kg/m^2)	26.73 ± 5.28	23.07 ± 3.71	<**0.001**
WC (cm)	92.2 ± 12. 61	85.35 ± 9.4	<**0.001**
WHR	0.91 ± 0.08	0.89 ± 0.04	**0.017**
WHtR	61.33 ± 9.89	54.18 ± 5.79	<**0.001**
Cholesterol (mg/dL)	212.83 ± 51.12	184.84 ± 61.76	<**0.001**
Triglycerides (mg/dL)	154.08 ±76.74	140.19 ± 32.43	**0.05**
HDL (mg/dL)	39.42 ± 13.56	44.36 ± 9.30	**0.001**
LDL (mg/dL)	142.58 ± 44.67	115.74 ± 59.22	<**0.001**
VLDL (mg/dL)	30.81 ± 15.34	28.56 ± 5.95	0.106
SBP (mmHg)	125.26 ± 10.00	123.88 ± 8.56	0.246
DBP (mmHg)	80.55 ± 5.29	78.79 ± 5.36	**0.011**
Fasting glucose (mg/dL)	94.89 ± 15.26	90.70 ± 12.58	**0.024**
Fasting Insulin (mU/L)	15.50 ± 5.78	11.84 ± 4.79	<**0.001**
Leptin (ng/ml)	17.02 ± 5.26	13.80 ± 2.44	<**0.001**
HOMA-IR	3.57 ± 1.62	2.73 ± 1.25	<**0.001**
QUICKI	0.49 ± 0.01	0.49 ± 0.01	<**0.001**
LH (mIU/ml)	12.57 ± 4.83	8.75 ± 4.67	<**0.001**
FSH (mIU/ml)	5.38 ± 1.86	5.87 ± 2.32	0.68
LH/FSH	2.51 ± 1.22	1.45 ± 0.36	<**0.001**
Testosterone (nmol/L)	2.10 ± 0.80	1.61 ± 0.42	<**0.001**

Values are expressed as mean ± SD (student t-test), $P \leq 0.05$ was considered as significant.
BMI=Body mass index, WC=Waist circumference, WHR=Waist-to-hip ratio, WHtR=Waist-to-height ratio, HDL=High-density lipoprotein, LDL=Low-density lipoprotein, VLDL=Very low-density lipoprotein, SBP: systolic blood pressure; DBP: diastolic blood pressure, HOMA-IR: homeostasis model for insulin resistance, QUICKI: quantitative insulin sensitivity check index, LH: luteinizing hormone, FSH: follicle-stimulating hormone, PCOS=Polycystic ovary syndrome

The PCOS and control groups were sub-grouped into obese/overweight (BMI ≥ 25) and normal/underweight (BMI< 25). Among anthropometric parameters, BMI and WHtR showed a significant difference in both normal/underweight and obese/overweight groups between PCOS and controls. Moreover, WC was found significantly increased in the normal/underweight PCOS group compared to the normal/underweight control group. Furthermore, fasting glucose and insulin, HOMA-IR, QUICKI, and serum leptin levels were significantly increased in the obese/overweight PCOS group compared to the obese/overweight control group (Table 2).

Table 2: Comparison of anthropometric and endocrine characteristics of PCOS and controls after subdividing into normal / underweight (BMI< 25) and obese / overweight (BMI ≥ 25) groups

Analyses	PCOS		Control		*P - value*
	normal/ underweight (n=70)	**obese/ overweight (n=80)**	**normal/ underweight (n=54)**	**obese/ overweight (n=46)**	
BMI (kg / m²)*†	22.20 ± 2.65	30.54 ± 3.73	20.01 ± 1.70	26.72 ± 1.50	**0.02**
WC (cm) †	90.78 ± 12.63	93.62 ± 11.98	81.50 ± 5.16	89.87 ± 9.21	**0.01**
WHR	0.90 ± 0.07	0.92 ± 0.08	0.89 ± 0.04	0.90 ± 0.04	0.58
WHtR *†	59.89 ± 10.24	62.60 ± 9.47	50.98 ± 4.45	57.93 ± 4.87	**0.04**
Cholesterol (mg / dL)	209.86 ± 51.82	215.42 ± 50.69	183.17 ± 68.24	186.81 ± 53.83	0.89
Triglycerides (mg / dL)	137.46 ± 60.08	168.62 ± 86.57	135.71 ± 26.95	145.45 ± 37.50	0.18
HDL (mg / dL)	42.25 ± 13.91	36.95 ± 12.82	45.21 ± 8.80	43.35 ± 9.86	0.26
LDL (mg / dL)	140.11 ± 44.62	144.74 ± 44.89	116.73 ± 68.18	114.57 ± 47.31	0.60
VLDL (mg / dL)	27.49 ± 12.01	33.72 ± 17.31	29.54 ± 4.86	29.75 ± 6.87	0.20
SBP (mmHg)	125.10 ± 11.49	125.40 ± 8.56	124.60 ± 8.20	123.04 ± 8.99	0.44
DBP (mmHg)	79.85 ± 5.53	81.17 ± 5.03	78.99 ± 5.07	78.54 ± 5.72	0.19
Fasting Glucose(mg / dL)*	93.95 ± 11.33	99.27 ± 15.52	92.82 ± 13.10	88.22 ± 11.59	**0.004**
Fasting Insulin (mU / L)*	13.37 ± 3.88	15.80 ± 5.88	12.10 ± 4.04	11.53 ± 5.57	0.02
HOMA-IR*	3.13 ± 1.10	3.95 ± 1.89	2.83 ± 1.15	2.60 ± 1.37	**0.006**
QUICKI *	0.49 ± 0.01	0.48 ± 0.01	0.49 ± 0.01	0.50 ± 0.01	**0.002**
Leptin (ng / ml)*	14.66 ± 4.58	19.08 ± 5.01	13.34 ± 2.56	14.33 ± 2.23	**0.001**
LH (mIU / ml)	11.91 ± 4.70	13.16 ± 4.91	8.72 ± 4.98	8.78 ± 4.33	0.33
FSH (mIU / ml)	5.26 ± 1.67	5.49 ± 2.0	5.60 ± 2.17	6.19 ± 2.47	0.49
LH / FSH	2.46 ± 1.34	2.56 ± 1.11	1.51 ± 0.44	1.38 ± 0.23	0.34
Testosterone (nmol / L)	1.96 ± 0.80	2.23 ± 0.79	1.50 ± 0.32	1.75 ± 0.49	0.89

Values are expressed as mean ± SD (two-way ANOVA analysis),$P \leq 0.05$ was considered as significant.

* shows significant differences between obese / overweight groups.† shows significant differences between normal / underweight groups.BMI=Body mass index, WC=Waist circumference, WHR=Waist-to-hip ratio, WHtR=Waist-to-height ratio, HDL=High-density lipoprotein, LDL=Low-density lipoprotein, VLDL=Very low-density lipoprotein, SBP: systolic blood pressure, DBP: diastolic blood pressure, HOMA-IR: homeostasis model for insulin resistance, QUICKI: quantitative insulin sensitivity check index, LH: luteinizing hormone, FSH: follicle-stimulating hormone, PCOS=Polycystic ovary syndrome

Pearson (r) correlation coefficient analysis was carried out for all anthropometric and endocrine parameters with serum leptin levels. Strong significant correlation was observed between serum leptin levels and BMI (*p-value* = < 0.001, r =0.50), LDL (*p-value* = 0.007, r =0.22), fasting glucose (*p-value* = < 0.001, r =0.31), fasting insulin (*p-value* = 0.006, r =0.22), HOMA-IR (*p-value* = < 0.001, r =0.31), cholesterol (*p-value* = 0.02, r =0.19), SBP (*p-value* = 0.01, r =0.19),DBP (*p-value* = 0.03, r =0.17) and significant negative correlation was found between serum leptin levels and QUICKI (*p-value* = < 0.001, r = - 0.32) in PCOS subjects (Figure 1). While in controls there was significant correlation between serum leptin levels with BMI (*p-value* = 0.03, r =0.21) and SBP (*p-value* = 0.01, r =0.23) (Figure 1). Other variables didn't show any significant correlation with serum leptin levels.

DISCUSSION

The endocrine and anthropometric parameters play a critical role in PCOS women, and any changes to those variables affect PCOS development. The present study showed that the anthropometric indices increased in the PCOS group compared to controls indicating total and central obesity are more prevalent in PCOS women when compared with control (Table 1). After subdividing to obese / overweight and normal / underweight groups, WC showed a significant difference between PCOS and control normal / underweight groups (Table 2). These results

explained that several PCOS subjects showed central obesity with normal BMI. Several studies have reported intra-abdominal fat has a stronger association with the risk of obesity-related morbidity than overall adiposity such as MetS and CVD (Ho et al., 2001; Guerrero-Romero and Rodríguez-Morán, 2003; Wang et al., 2017).

Our findings showed that fasting glucose, insulin, and insulin resistance increased in PCOS obese/overweight groups compared to controls. This results indicate that, high level of fasting glucose, insulin and insulin resistance, related to obesity and not solely due to PCOS. Abdominal obesity leads to increased insulin levels (Tucel et al., 2006), and the subsequent hyperinsulinemia may stimulate additional obesity (Dunaif and Book, 1997). Furthermore, OUICKI decreased in PCOS obese/overweight group compared to the control group. Any degree of obesity is likely to result in decreased insulin sensitivity.

As a primary endocrine organ, adipocytes produce leptin. Leptin has been recognized as a key protein in the weight-control system, and its absence leads to increased appetite, reduced energy expenditure, and, ultimately, obesity (Abdella et al., 2005). Serum leptin levels significantly were increased in PCOS obese/overweight group compared to control group. Serum leptin levels studies in women with PCOS showed contradictory results. We found an elevated amount of serum leptin in PCOS patients, similar to some previous studies (Mitkov et al., 2008; Pehlivanov and Mitkov, 2009; Chakrabarti et al., 2013; Kumawat et al., 2021). Other studies have been unable to find any differences between PCOS and healthy women (Glintborg et al., 2006; Bideci et al., 2008). Differential findings can be attributed to variations in age or the severity of the condition.

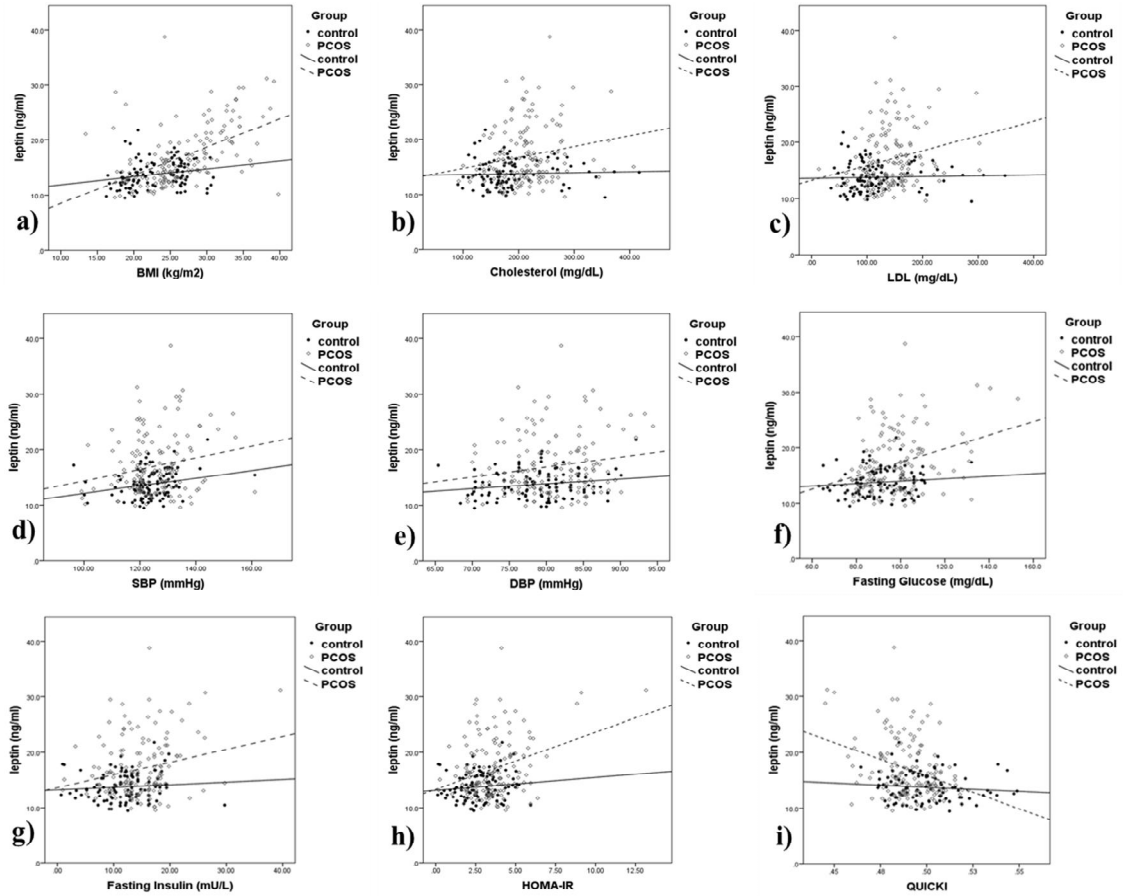

Figure 1: Linear regression presenting the associations of leptin with a) BMI, b) Cholesterol, c) LDL, d) SBP, e) DBP, f) Fasting Glucose, g) Fasting Insulin, h) HOMA-IR, i) QUICKI

Our findings showed a strong correlation between serum leptin levels and BMI in PCOS and controls (Figure 1), corroborating previous studies (Kumawat et al., 2021; Glintborg et al., 2006; Cheng et al., 2014; Jahromi et al., 2017). Logically, enhancement in fat cells and BMI should be followed by an increase in serum leptin production. In PCOS women, dyslipidemia and obesity are frequent metabolic abnormalities (Thathapudi et al., 2014; Layegh et al., 2016), so reasonably, in the present study; there was a significant correlation between serum leptin with cholesterol and LDL in PCOS subjects, not controls. Also, a previous study on Saudi PCOS women reported the same correlation between serum leptin and lipids (Daghestani et al., 2019).

Furthermore, this study demonstrated a significant correlation between serum leptin levels with fasting glucose, insulin, and HOMA-IR and a negative correlation with QUICKI (Figure 1). Several prior studies reported a significant correlation of serum leptin levels with insulin (Chakrabarti et al., 2013; Daghestani et al., 2019), insulin resistance (Jahromi, 2017; Mohiti et al., 2009), and fasting glucose (Jahromi, 2017). In contrast, Jalilian et al. found no significant correlation of serum leptin levels with insulin and fasting glucose in Iranian women with PCOS (Jalilian et al., 2016). Insulin has been found to increase leptin mRNA directly in adipocytes in vitro, indicating that insulin may stimulate leptin release (Abdella et al., 2005; Stefanović et al., 2008). Moreover, the study's significant correlation between serum leptin levels and SBP and DBP is consistent with observational findings of the association of leptin with CVD (Li et al., 2011). The major mechanisms through which leptin trigger CVD are its associations with high blood pressure and pro-atherogenic blood lipids and its role in endothelial dysfunction and insulin resistance (Li et al., 2011).

The insignificant correlation between serum leptin levels and testosterone in this study is in agreement with previous studies (Chakrabarti, 2013; Kumawat et al., 2021; Glintborg et al., 2006; Saleh et al., 2004). Leptin may influence androgen levels by affecting insulin production rather than directly altering blood testosterone levels (Houjeghani et al., 2012).

The limitation of the present study was the relatively small sample size. Also, the studied PCOS women were confined to a certain geographical area. Therefore, future studies should replicate the investigation in a larger sample size and various populations to confirm our results. However, investigation of endocrine and anthropometric parameters in subdivided obese/overweight and normal/ underweight groups have been considered as the strength of the current study.

CONCLUSION

In conclusion, serum leptin levels showed a strong correlation with MetS risk factors, especially glucose metabolic abnormality in PCOS women compared to control. Mutual association of BMI with PCOS and leptin leads to the association of leptin levels with metabolic risk factors, which are frequently, presented in PCOS women. Our findings showed a weak correlation between serum leptin levels with reproductive hormones in studied subjects.

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Conflict of interest

There are no conflicts of interest

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