

Research Article

A study on assessment of serum Leptin and serum Insulin in women with polycystic ovarian syndrome

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Abstract

Background: Polycystic ovarian syndrome is characterized by chronic anovulation, hyperandrogenism and ovarian polycystic changes on ultrasonogram. PCOS is often accompanied by obesity and insulin resistance. Leptin is produced mainly by the adipocytes. Debatable reports of relationship between leptin and insulin resistance exist. The objectives of study were to assess serum leptin and insulin level in PCOS subjects and to find out the correlation between serum leptin level and BMI in PCOS subjects and controls.

Methodology: The study was carried out in 30 PCOS subjects between the age group of 18- 35 years and 30 age and BMI matched healthy women with normal menstrual cycle as controls. Fasting blood samples were collected. Serum leptin and serum insulin were estimated by ELISA and ECLIA methods respectively. Insulin resistance was calculated by HOMA. FBS, blood urea and serum creatinine were also measured.

Results: Serum Insulin and HOMA-IR were significantly high ($p < 0.001$) in PCOS subjects compared to controls. Higher mean serum leptin, mean BMI and mean FBS were observed in the PCOS subjects compared to controls but the difference between the two groups were not statistically significant. Leptin showed a significant positive correlation with BMI in the PCOS subjects and controls.

Conclusion: PCOS is associated with insulin resistance and places the subject at a higher risk of metabolic syndrome. Elevated serum leptin in PCOS women appears to be due to the positive correlation between leptin and BMI. Thus follow-up of these subjects with regard to metabolic syndrome may be beneficial.

Keywords: Polycystic ovarian syndrome, Serum Leptin, Serum Insulin.

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disease and metabolic disorder in adolescence and reproductive women, which is the first reason for female infertility, with the incidence of 5–10% in reproductive women.¹ According to ESHRE/ASRM consensus workshop at Rotterdam in 2003, the diagnosis of PCOS is based on the presence of any two of (1) chronic anovulation, (2) clinical/biochemical parameters for hyperandrogenism, and (3) polycystic ovaries on ultrasonography.² PCOS subjects are often accompanied by obesity, insulin resistance, abnormal glucose metabolism, lipid disorder, hypertension, and other risk factors of cardiovascular disease.¹ The pathophysiology is complex involving the hypothalamus-pituitary-ovarian axis, ovarian theca cell hyperplasia, hyperinsulinemia and a multitude of other cytokine and adipocyte-driven factors.³

Leptin, a 167-amino acid protein, is the gene product of the *ob* gene predominantly produced by adipocytes. It is a polypeptide hormone essential in the regulation of normal body weight. The production of leptin is under neuroendocrine control and leptin receptors are found in various endocrine organs, including the pancreas and ovaries.^{4,5} Leptin has been shown to improve insulin sensitivity and glucose metabolism and inhibit insulin secretion. However studies are there showing that circulating leptin can counteract the effect of insulin in both muscle and adipocytes thus inducing peripheral insulin resistance. Therefore it is evident from previous studies that influence of leptin on insulin action is debatable.⁶ Serum leptin levels are increased in obesity, being strongly associated with fat mass. This indicates that obesity is a leptin resistant state in most obese individuals rather than it being a condition of defective *ob* gene.^{7,8} Data from experiments in isolated adipocytes and from clinical studies in human subjects support the idea that insulin increases leptin production indirectly via its effects to increase glucose utilization and oxidative glucose metabolism in adipocytes at the transcriptional level.⁹ The objectives of the study was to assess serum leptin and serum insulin level in PCOS subjects and to find out the correlation between serum leptin level and BMI in subjects of PCOS and controls.

2. Materials and Methods

The study was carried out on 30 PCOS subjects in the age group of 18 to 35 years and 30 voluntary age and BMI matched healthy women with normal menstrual cycle as controls. The study was conducted at Kempegowda Institute of Medical Sciences & Hospital. The diagnosis of PCOS was fulfilled as per Rotterdam criteria. Presence of at least two criteria from clinical, hormonal and abdominal USG category was considered diagnostic of PCOS. Patients with diabetes mellitus, hypertension, dyslipidemia, renal and liver failure and other endocrine disorders and patients receiving hormonal / non-hormonal treatment for PCOS were excluded from the study. The institutional ethical committee approved the study protocol. Informed consent was obtained from all the participants.

A pre-structured and pre-tested proforma was used to collect the data. Baseline data including age, BMI, detailed medical history, clinical examinations and relevant investigations were included as part of the methodology. Serum leptin, serum insulin, blood sugar, blood urea and serum creatinine were measured in all participants from morning blood samples collected after 12 hours of fasting. Serum leptin was measured by Sandwich ELISA method (Diagnostic Biochem Canada Inc. Cat. No. CAN-L-4260; Version:8.1; August 2009). Serum insulin was measured by electrochemiluminescence immunoassay (Elecsys 2010 analyzer, Roche Diagnostics). IR was estimated via the homeostasis model

assessment insulin resistance index (HOMA-IR), as follows: $\text{HOMA-IR} = \text{fasting insulin (mU/L)} \times \text{fasting glucose (mmol/L)} / 22.5$. Body mass index (BMI) was calculated as the ratio of weight (Kg) to height squared (m^2). Blood sugar was estimated by GOD/POD method, blood urea was estimated by Specific urease/ glutamate dehydrogenase method and serum creatinine was estimated by Jaffe's Alkaline Picrate Method.

2.1 Statistics analysis

SPSS software version 13.0 was used for statistical analysis. Comparisons between groups were performed using the Mann-Whitney test. Correlation analysis between BMI, serum leptin and serum insulin were done using Spearman's rank order correlation coefficients. A P value < 0.05 was considered statistically significant.

3. Results

Results on continuous measurements are presented as Mean \pm SD. The basic characteristics of the cases and controls are depicted in Table 1. There was no significant difference in age between two groups. Slightly higher mean BMI was recorded in cases than in controls but the difference in mean BMI between the two groups was not statistically significant ($P > 0.05$). The mean distributions of serum leptin, serum insulin, HOMA-IR, FBS, urea and creatinine levels are depicted in Table 2. Higher mean fasting serum Insulin and higher mean HOMA-IR were recorded in cases compared to controls and the difference between them were found to be statistically significant ($P < 0.001$). No significant correlation could be found between BMI and serum Insulin in cases ($\rho = 0.283$, $p = 0.130$) or controls ($\rho = -0.163$, $p = 0.388$). Higher mean leptin and FBS were recorded in cases compared to controls but differences between cases and controls were not statistically significant ($P \geq 0.05$). Correlation of leptin with insulin and BMI is depicted in Table 3. Significant positive correlation between leptin levels and BMI in cases and controls ($\rho = 0.683$, $p < 0.001$; $\rho = 0.485$, $p = 0.007$ respectively) was found in our study, which is shown in figure 1. No significant correlation could be found between Leptin and fasting serum Insulin in cases ($\rho = 0.289$, $p = 0.121$) or controls ($\rho = -0.080$, $p = 0.673$).

Table 1: Basic characteristics of study population

Parameters	Cases with PCOS (n=30)	Controls (n = 30)	P value
Age (years)	23.37 \pm 4.09	23.73 \pm 3.81	0.744
BMI ((kg/m ²))	24.00 \pm 4.41	22.51 \pm 2.31	0.126

Values are expressed as means \pm SD.

Table 2: Mean distribution of biochemical parameters in PCOS cases and controls.

Parameters	Cases with PCOS (n=30)	Controls (n = 30)	P value
Serum Leptin	10.61 \pm 12.52	9.01 \pm 4.87	0.813
Serum Insulin	12.01 \pm 6.74	6.80 \pm 3.10	$<0.001^*$
HOMA-IR	2.35 \pm 1.40	1.27 \pm 0.58	$<0.001^*$
FBS	80.33 \pm 10.53	74.67 \pm 9.59	0.050
Blood urea	17.86 \pm 4.35	17.77 \pm 3.91	0.458
Serum creatinine	0.66 \pm 0.10	0.66 \pm 0.12	0.697

Values are expressed as means \pm SD.

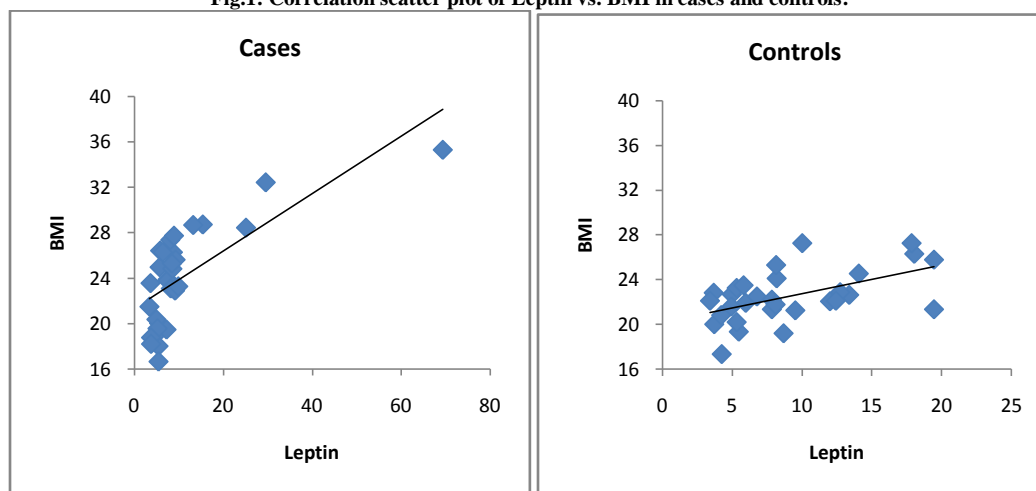
*denotes significant difference

Table 3: Correlation between various parameters

Parameters	Cases		Controls	
	ρ value	P value	ρ value	P value
Leptin and BMI	0.683	$< 0.001^*$	0.485	0.007*
Leptin and Insulin	0.289	0.121	-0.080	0.673
BMI and Insulin	0.283	0.130	-0.163	0.388

*denotes significant correlation

Fig.1: Correlation scatter plot of Leptin vs. BMI in cases and controls:



4. Discussion

The consequences of the polycystic ovary syndrome extend beyond the reproductive axis; women with the disorder are at substantial risk for the development of metabolic and cardiovascular abnormalities. Both PCOS and the metabolic syndrome share insulin resistance as a pathogenic feature. The incidence of impaired glucose tolerance, type 2 diabetes mellitus, obesity, hypertension, and dyslipidemia, as well as of coronary and vascular disease, may be higher in women with PCOS during their reproductive years.³

Higher mean FBS was recorded in PCOS subjects compared to controls in our study but the difference in mean FBS between the two groups was not statistically significant ($P \geq 0.05$). Dunaif *et al* found in their study that the obese PCOS women had significantly increased fasting glucose levels compared with their body-composition matched control group, resulting from small but significant increases in basal hepatic glucose production.¹⁰ In our study, higher mean Fasting Serum Insulin was recorded in PCOS subjects compared to controls and the difference between them was statistically significant ($P < 0.001$). We could not find any significant correlation between BMI and serum insulin level in either of the groups. Insulin resistance is defined clinically as the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization in an individual as much as it does in a normal population.¹ In our study, higher mean HOMA-IR was recorded in PCOS subjects compared to controls and the difference between them was found to be statistically significant ($P < 0.001$). This was consistent with Shou-Kul Xiang *et al*. They found in their study that the HOMA-IR of the PCOS women was significantly higher than that of the age-matched healthy women, which suggested that insulin resistance had a crucial role in pathogenesis of PCOS.¹

Chang *et al*. described insulin resistance also in non-obese women with PCOS. They showed in their study that mean basal insulin levels and insulin responses to glucose administration in PCOS subjects were significantly greater than those found in normal control subjects indicated the presence of insulin resistance in the absence of obesity. Furthermore, their results indicated a strong positive correlation between circulating androgen levels and insulin secretion. These observations suggest that hyperandrogenism may be, in part, responsible for the insulin resistance in PCOS.¹¹ Burghen *et al* also demonstrated a striking positive correlation between hyperandrogenism and hyperinsulinism in PCOS with relatively mild glucose intolerance.¹² Puder *et al* showed in their study that women with PCOS were more insulin resistant compared to a group of age and BMI matched controls.¹³

The impact of obesity is usually considered to operate through the associated IR. Dunaif and her colleagues have described a specific defect in transduction of the insulin signal (autophosphorylation of the serine rather than tyrosine residues of the intracellular component of the insulin receptor) creates IR which is considered to be a constitutive feature of fibroblasts of women with PCOS. Obesity itself, present in some 40% of women with PCOS, worsens IR and so causes further deterioration of ovarian function.¹⁴ Insulin can stimulate ovarian androgen production in normal women and in women with PCOS. However, ovarian cells of women with PCOS display a higher responsiveness for insulin stimulated androgen synthesis *in vitro*. It is therefore, probable that women develop PCOS because of a hypersensitivity of their intraovarian insulin androgen signaling pathway.¹⁵

Serum leptin is found to be keenly interrelated with estrogens, progesterone, androgens, and insulin. PCOS features are often linked to leptin and its receptor. These facts thus make PCOS women the useful subjects to assess the interregulatory phenomena between leptin and ovarian function. Expression of leptin receptors in granulosa cells, which synergies with glucocorticoids to promote steroidogenesis, indicates that leptin exerts direct regulatory action in ovarian folliculogenesis.¹⁶ In our study, higher mean serum leptin was recorded in PCOS subjects compared to controls but the difference between them was not statistically significant ($P > 0.05$). The results of the present study showed that a significant positive correlation exists between serum leptin and BMI both in PCOS subjects ($p < 0.001$) and controls ($p = 0.007$) suggesting that leptin is secreted from adipocytes into circulation and by acting as a sensing hormone to hypothalamus informing the brain about abundance of body fat. This finding was consistent with study done by Javed Mohiti-Ardekani *et al*¹⁷, Tayfun Alper *et al*⁴ and Chakrabarti J¹⁶. Study done by Javed Mohiti-Ardekani *et al* showed a significant high total and free leptin in PCOS women as compared to controls and total leptin levels correlated significantly with BMI in both PCOS women and controls.¹⁷ Tayfun Alper *et al* observed in their study that serum leptin levels as well as basal insulin levels, IR and BMI were significantly high in PCOS women. Although leptin production mainly occurs in adipose tissue, when the difference in body fat mass between PCOS and controls was corrected for, the difference in the leptin levels remained significant. This finding suggested that there might be other reasons for the increase in the serum leptin concentration in PCOS cases.⁴ Study done by Chakrabarti J showed that irrespective of BMI or insulin resistance, PCOS population had higher leptin levels. This observation is because leptin is predominantly synthesized by adipocytes, and higher BMI is observed in the PCOS group than in control women.¹⁶ In contrast, Rouru *et al* found in their study that serum leptin concentrations were not significantly different in PCOS and control subjects.¹⁸ Laughlin *et al* also found that leptin levels in PCOS did not differ from those of normal cycling women with similar BMI or adiposity.¹⁹

Insulin has been shown to increase leptin mRNA in adipocytes, suggesting its possible role in stimulating leptin secretion. It may be that the elevated leptin in hyperinsulinemic PCOS women is a secondary consequence of insulin stimulated synthesis of leptin. Insulin plays a chronic role in the regulation of leptin gene expression and production by white adipose tissue. Insulin appears to act directly at the level of the adipocyte by increasing leptin secretion and gene expression, perhaps due to increased glucose transport and metabolism.²⁰ Leptin on the other hand, inhibits insulin mediated promotion of gonadotropin stimulated steroidogenesis. There are reports that leptin decreases glucose mediated insulin secretion through its receptors in the hypothalamus and also attenuates its action at the cellular level.¹⁶ Leptin is one of the factors of relationship between obesity and insulin resistance and between obesity and DM2. It has been proven that on rat's adipocytes, leptin lessens the insulin bond with its receptors, thus fomenting insulin resistance. Both leptin and insulin regulate themselves one another. Thus, leptin inhibits insulin production on pancreas β cells, while insulin entices leptin production on the adipose tissue. So it is worth posing that hyperleptinemia might be a crucial factor upon insulin resistance regarding most on obese patients.²¹ Javed Mohiti-Ardekani *et al* in their study showed that Leptin correlated with insulin resistance and insulin level in both cases and controls.¹⁷ El-Gharib *et al* also found the significant positive correlation between serum insulin and leptin levels in PCOS subjects.¹⁵ In our study, no significant correlation could be found between serum leptin and serum insulin in either of the groups. This finding was mostly because our study consisted of limited number of subjects.

Our study implicates the utility of BMI, serum leptin and fasting serum insulin in PCOS subjects for evaluating risk of metabolic syndrome which would be helpful for an early medical intervention.

5. Conclusion

The parameters such as BMI, FBS and leptin were elevated in the PCOS subjects compared to controls. Fasting Serum Insulin and HOMA-IR were found to be significantly higher in PCOS subjects compared to controls. There was a significant positive correlation between BMI and leptin in PCOS subjects and controls.

All the above derangements confirm that PCOS is associated with insulin resistance and places the subject at a higher risk of metabolic syndrome. Elevated serum leptin in polycystic ovarian women appears to be due to the positive correlation between leptin and BMI. Taken together the present observations made in this study propose that regulation of leptin synthesis and its action on female reproductive system are governed by a plethora of controlling factors. Thus follow-up of these patients with regard to the development of diseases associated with metabolic syndrome may be beneficial.

Because our study consisted of a limited number of PCOS subjects and controls from a single population, further studies with larger number of PCOS subjects will be beneficial in elucidating the relationship between leptin, serum insulin and BMI in polycystic ovarian syndrome.

References

1. Shou-Kul Xiang, Fei Hua, Ying Tang, Xiao-Hong Jiang, Qi Zhuang, Feng-Juan Qian. Relationship between serum lipoprotein ratios and insulin resistance in polycystic ovary syndrome. *International Journal of Endocrinology*. 2012 April; 2012(2012), 173281:1-4.

2. Pikee Saxena, Anupam Prakash, Aruna Nigam, Archana Mishra. Polycystic ovary syndrome: Is obesity a sine qua non? A clinical, hormonal, and metabolic assessment in relation to body mass index. *Indian Journal of Endocrinology and Metabolism*. 2012 Nov-Dec; 16(6):996-9.
3. Carolyn J. Alexander, Edward P. Tangchitnob, Norman E. Lepor. Polycystic Ovary Syndrome: A Major Unrecognized Cardiovascular Risk Factor in Women. *Med Reviews*. 2009; 2(4).
4. Tayfun Alper, Hakki Kahraman, Mehmet Bilge Cetinkaya, Filiz Yanik, Gulizar Akcay, Abdulkirim Bedir et al. Serum leptin and body composition in polycystic ovarian syndrome. *Ann Saudi Med*. 2004 January-February; 24(1):9-12.
5. Vicken P. Sepilian, John R. Crochet, Manubai Nagamani. Serum soluble leptin receptor levels and free leptin index in women with polycystic ovarian syndrome: relationship to insulin resistance and androgens. *Fertility and Sterility*. 2006 May; 85(5).
6. Khatri Anshu, Aggarwal Tanu, Changotra Prashant, Srivastava Neena, Tiwari Sunita, Mishra Payal. Plasma leptin levels and body mass index in north Indian subjects: correlation with insulin resistance. *Journal of Advance Researches in Biological Sciences*. 2013; 5(1):59-62.
7. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR et al. Serum immunoreactive – leptin concentrations in normal weight and obese humans. *New Engl J Med*. 1996; 334:292 - 5.
8. Mantzoros CS. The role of leptin in human obesity and disease: a review of current evidence. *Ann Intern Med*. 1999; 130:671-80.
9. Peter J. Havel. Regulation of Energy Balance and Carbohydrate/Lipid Metabolism. *Diabetes*. 2004 February; 53(1):S143–S151.
10. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes*. 1989; 38:1165-74.
11. Chang RJ, Nakamura RM, Judd HL, Kaplan SA. Insulin resistance in nonobese patients with polycystic ovarian disease. *J Clin Endocrinol Metab*. 1983; 57:356-9.
12. Burghen GA, Givens JR, Kitabachi AE. Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. *J Clin Endocrinol Metab*. 1980; 50:113-6.
13. Puder JJ, Varga S, Kraenzlin M, et al. Central fat excess in polycystic ovarian syndrome: relation to low-grade inflammation and insulin resistance. *J Clin Endocrinol Metab*. 2005; 90:6014-21.
14. Andrea Dunaif, Jinru Xia, Carol-Beth Book, Esther Schenker, Zhichun Tang. Excessive Insulin Receptor Serine Phosphorylation in Cultured Fibroblasts and in Skeletal Muscle: A Potential Mechanism for Insulin Resistance in the Polycystic Ovary Syndrome. *J. Clin. Invest*. 1995; 96:801-10.
15. El-Gharib MN, Badawy TE. Correlation between insulin, leptin and polycystic ovary syndrome. *J Basic Clin Reprod Sci*. 2014; 3:49-53.
16. Chakrabarti J. Serum leptin level in women with polycystic ovary syndrome: Correlation with adiposity, insulin, and circulating testosterone. *Ann Med Health Sci Res*. 2013; 3:191-6.
17. Javed Mohiti-Ardekani, Nasim Tarof, Abbas Aflatonian. Relationship between free leptin and insulin resistance in women with polycystic ovary syndrome. *Iranian Journal of Reproductive Medicine*. 2009 spring; 7(2):53-8.
18. Rouru J, Anttila L, Koskinen P, Penttila TA, Irjala K, Huupponen R, et al. Serum Leptin Concentrations in Women with Polycystic Ovary Syndrome. *J Clin Endocrinol Metab*. 1997; 82:1697-700.
19. Laughlin G. A., Morales A. J., Yen S. S. C. Serum Leptin Levels in Women with Polycystic Ovary Syndrome: The Role of Insulin Resistance/ Hyperinsulinemia. *Journal of Clinical Endocrinology and Metabolism*. 1997; 82(6):1692-96.
20. Margetic S, Gazzola C, Pegg GG, Hill RA. Leptin: a review of its peripheral actions and interactions. *International Journal of Obesity*. 2002; 26:1407–33.
21. Greenfield JR, Campbell LV. Insulin resistance and obesity. *Clin Dermatol*. 2004; 22:289-95.