

Original Research Article

TO EVALUATE THE ASSOCIATION BETWEEN SERUM LEPTIN AND INSULIN/INSULIN RESISTANCE IN POLYCYSTIC OVARIAN SYNDROME IN A TERTIARY CARE CENTRE IN NORTH INDIA.

Abstract

Background

Polycystic ovarian syndrome (PCOS) a common endocrinological disorder among women in the reproductive age group, is associated with hyperinsulinemia/Insulin resistance, obesity, menstrual irregularities and anovulation.

The present study was conducted to evaluate the association between insulin/insulin resistance and leptin among women with PCOS in a sample population of a tertiary care centre in North India.

Materials – Ninety newly diagnosed cases of PCOS as per Rotterdam criteria were enrolled in the study, along with ninety normal women of similar age and BMI. Serum insulin, fasting glucose and serum leptin were estimated, Insulin resistance was calculated using (HOMA-IR) the homeostatic model for assessment-insulin resistance.

Results- In our study leptin levels were raised in both cases and in controls. Leptin levels correlated positively with BMI among the cases ($r=0.21$) and also among the controls ($r=0.003$). In our study PCOS women had higher mean BMI and leptin levels when compared to controls. Insulin levels correlated positively with BMI among the cases ($r=0.21$) and was statistically significant ($P=.004$). Insulin levels were higher in women with BMI in the overweight and obese category. Although most of our cases were of lean weight and BMI in the normal range, high insulin and IR was detected in them showing impaired glucose metabolism. Leptin levels were higher in cases group who also had high IR when compared to healthy controls in our study. Leptin levels correlated positively with IR among cases ($r=0.25$) and this was statistically significant ($P=.013$).

Conclusion- Our study showed higher serum Leptin, insulin and IR among PCOS cases when compared to the control group.

Keywords- IR- Insulin resistance , Leptin, BMI- body mass index.

Introduction

Polycystic ovarian syndrome is one of the most common endocrine disorder, seen in about 20% of women in the reproductive age group. The symptoms seen in majority of patients are a combination of hirsutism, acne, menstrual disturbances leading to anovulation and infertility. Obesity is also seen in PCOS quite commonly. The possible long term effects include type 2 diabetes mellitus, hypertension and cardiovascular disease. PCOS is a state of hyperinsulinemia and insulin resistance.¹

Leptin secreted by adipose tissue is a peptide hormone, a product of the OB gene. It plays an important role in controlling appetite and energy balance. The relationship between leptin and obesity is well known. It has been shown that insulin directly induces leptin mRNA synthesis in adipocytes in rats, suggesting that insulin may increase leptin secretion. The etiology of PCOS is not known. The presence of obesity, hyperandrogenemia, insulin resistance and infertility in PCOS resemble those in leptin deficient rats in invitro studies.^{2,3} This led to this study being designed to evaluate the association of insulin resistance, insulin levels in PCOS cases with serum leptin concentration in a tertiary care centre of North India.

Materials and methods

The present study was conducted in the Department of Biochemistry, Pt.B.D.Sharma PGIMS, Rohtak, in collaboration with the Endocrinology clinic of the Department of Medicine, Pt.B.D.Sharma PGIMS Rohtak.

Ninety newly diagnosed cases of PCOS women in the reproductive age (18-40)years attending the Department of Medicine for treatment and satisfying the inclusion and exclusion criteria were enrolled in the study.

Ninety Healthy age matched female volunteers were enrolled as controls.

Inclusion criteria

Newly diagnosed female patients with PCOS as per Rotterdam criteria, of reproductive age group mentioned above and before starting any treatment.

Exclusion criteria

Some other potential causes of hyperandrogenism, oligo/amenorrhea, such as congenital adrenal hyperplasia, androgen secreting tumour, hypothyroidism, Cushing's syndrome, hyperprolactinemia and other insulin resistance conditions like Acromegaly.

Methodology

A written consent was obtained from all the patients participating in the study. The clinical details from all the patients was recorded in proforma. Routine biochemical, hormonal investigations and ultrasonography was carried out.

Leptin estimation was done by ELISA using leptin ELISA kit⁴. Insulin estimation was done by immuno assay.⁴

Insulin resistance was calculated using the homeostatic model assessment insulin resistance (HOMA-IR).

HOMA-IR was calculated as the product of the insulin value ($\mu\text{U/ml}$) and the fasting plasma glucose value (mg/dl), divided by 405.

Sample collection and processing

Four ml of venous blood sample was taken from the antecubital vein aseptically in plain red vacutainer for leptin, insulin and other routine biochemical analysis. Serum was separated by centrifugation at 2000 rpm for 10 minutes after clotting. Separated serum was stored at -20°C till further analysis.

Results

Thirty two women (35.5%) diagnosed with PCOS were in the age group 18-20 years while 28(31.1%) women were in the age group 21-25 years. Fourteen (15.5%) women were in the age group 26-30 years and sixteen (17.7%) women were in the age group 31-40 years. In

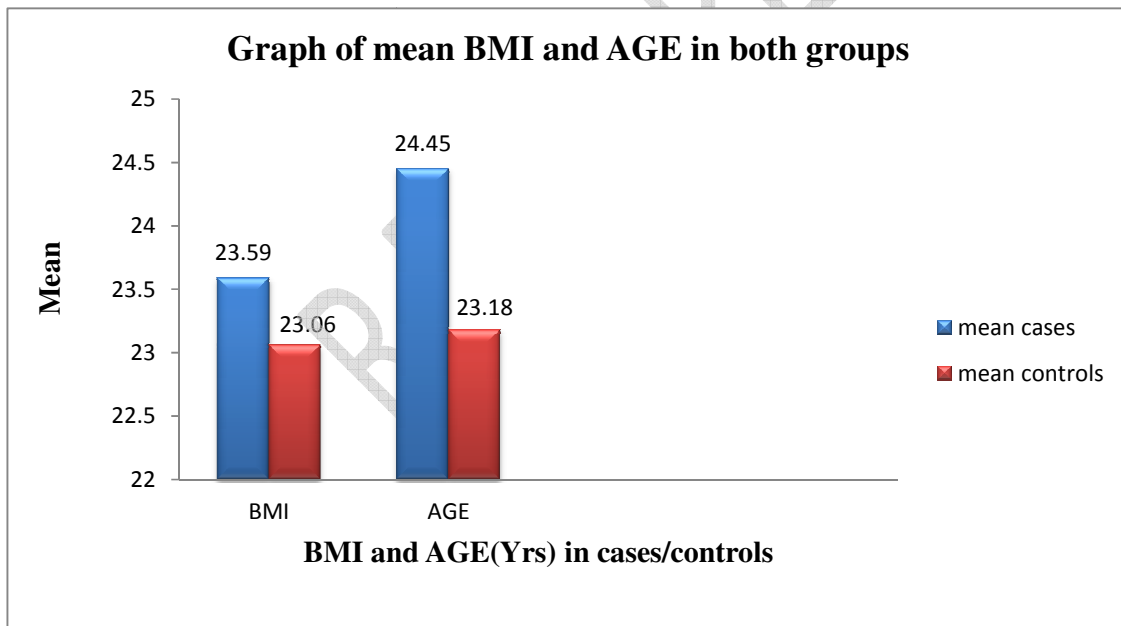
group 2 or in the controls sixty eight (75.5%) women were in the age group 21-25 years while only eleven (12.2%) women were in the 18-20 years age group. There were only ten(11.1%) and one(1.1%) women in the age groups 26-30 years,31-40 years respectively among the controls.

The mean and SD of age of cases (group 1) was 24.45 ± 6.16 years .

The mean and SD of age of controls (group 2) was 23.18 ± 2.26 years.

In the present study the mean BMI among cases was 23.59 and the mean BMI among controls was 23.06. The distribution of BMI among cases was such that 62 (68.8%) women had BMI in the range 18-24 that is they were in the normal range. Seventeen (18.8%) women had BMI in the range 24-30 that is they were overweight and eight (8.8%) of women had BMI above 30 that is they were obese.

Graph1



Serum Insulin levels were significantly decreased in the control group when compared to the cases group ($P=.01$). The Insulin resistance was calculated using HOMA-IR formula. Insulin resistance was compared between both groups. Insulin resistance was significantly raised in the cases group when compared with the control group. ($P=.02$). Insulin resistance showed a positive correlation with BMI in both the groups. Serum Insulin levels showed a positive correlation with BMI in both groups. The correlation between serum insulin and BMI in the

cases group was statistically significant ($P=.004$), whereas the correlation between serum insulin and BMI in the controls group was statistically insignificant ($P=.64$).

Serum Leptin levels were comparable between cases and controls but the difference was not statistically significant ($P=.06$). Serum Leptin levels showed a positive correlation with BMI in both groups and these correlations were statistically significant in both groups. Serum leptin levels showed a positive correlation with insulin resistance in both the groups. The correlation between serum leptin and insulin resistance in the cases group was statistically significant ($P=.013$) but the correlation between serum leptin and insulin resistance in the control group was statistically insignificant ($P=.80$).

Discussion

Polycystic ovarian syndrome is now recognised as an important metabolic as well as a reproductive disorder in women, with long term sequelae which include diabetes mellitus, hypertension and heart disease. PCOS is characterised by menstrual disturbances, clinical and biochemical manifestations of hyperandrogenism and polycystic ovaries.¹

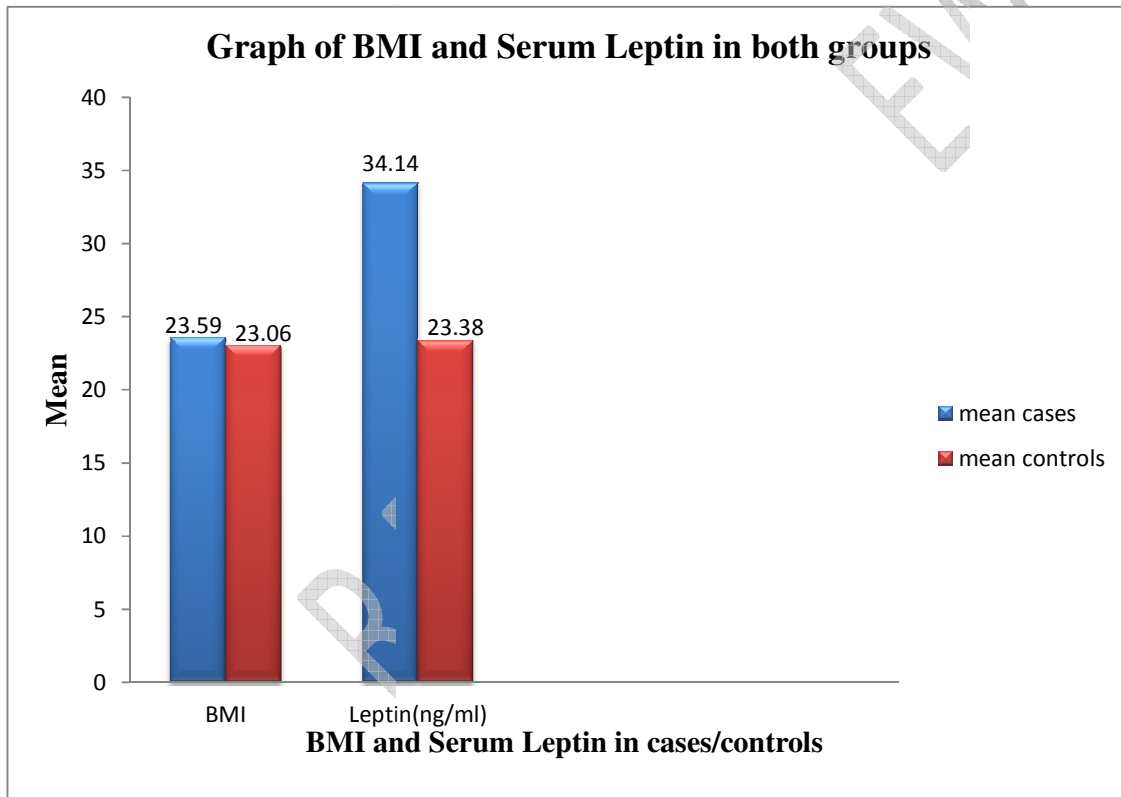
Around 60% of women with PCOS are hirsute, the most common clinical sign of hyperandrogenemia.⁵ The discovery of leptin and its association with energy balance and reproductive axis, has led to considerable interest in association between PCOS and leptin levels. Hence our study was planned to find the correlation between insulin, leptin and insulin resistance in women with PCOS in a tertiary care centre in north India.

In the present study around 35.5% of women diagnosed with PCOS were in the age group of 18 to 20 years, while 31.1% of women with PCOS were in the age group 21 to 25 years. This finding of higher prevalence of PCOS in adolescent girls and young women in this region is consistent with findings from a community study on PCOS prevalence in North India by Gill H et al.⁶ The mean age of cases was 24.4 years. (Graph 1).

In the present study the mean BMI among cases was 23.59 and the mean BMI among controls was 23.06. The distribution of BMI among cases was such that 62 (68.8%) women had BMI in the range 18-24 that is they were in the normal range. Seventeen (18.8%) women had BMI in the range 24-30 that is they were overweight and eight (8.8%) of women had BMI above 30 that is they were obese. Studies by Gill et al⁶ and Joshi et al⁷ showed higher

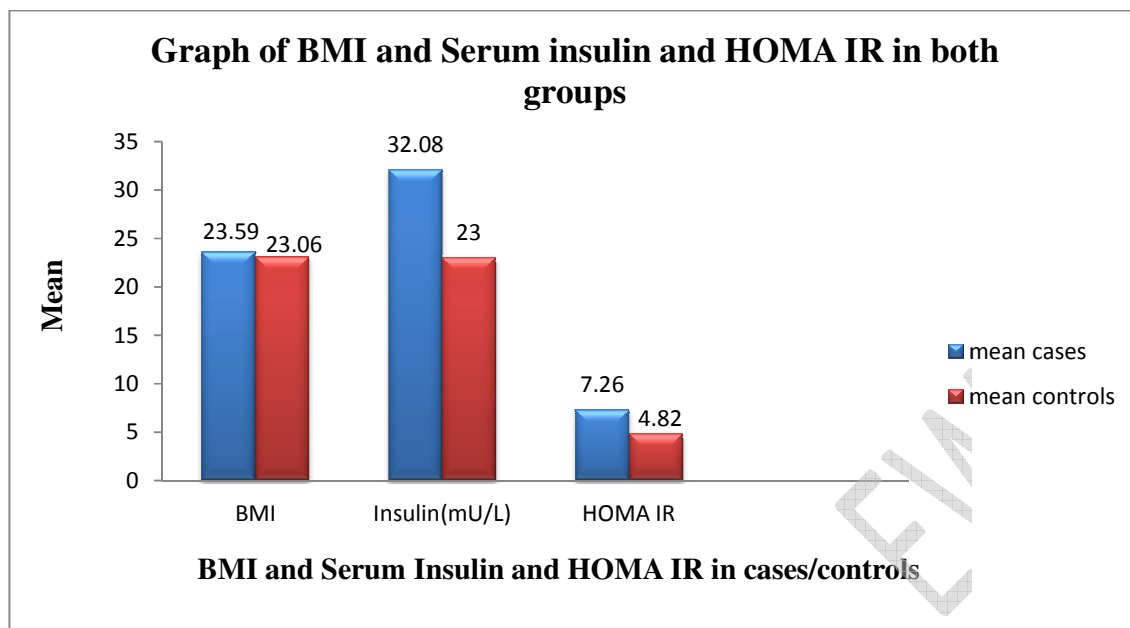
rates of PCOS among young women with BMI in the non-obese range. This is consistent with the findings in our study that majority of women diagnosed with PCOS were of lean weight and non-obese. In the present study the mean insulin levels among cases and controls were comparable, but not significant. The mean insulin level in cases was (32.08 ± 19.09) mU/L and in controls was (23.0 ± 4.74) mU/L. Our study showed that insulin levels in PCOS women and among healthy age matched women were not that significantly different. Our study also showed hyperinsulinemia in both lean and obese PCOS cases.

Graph2

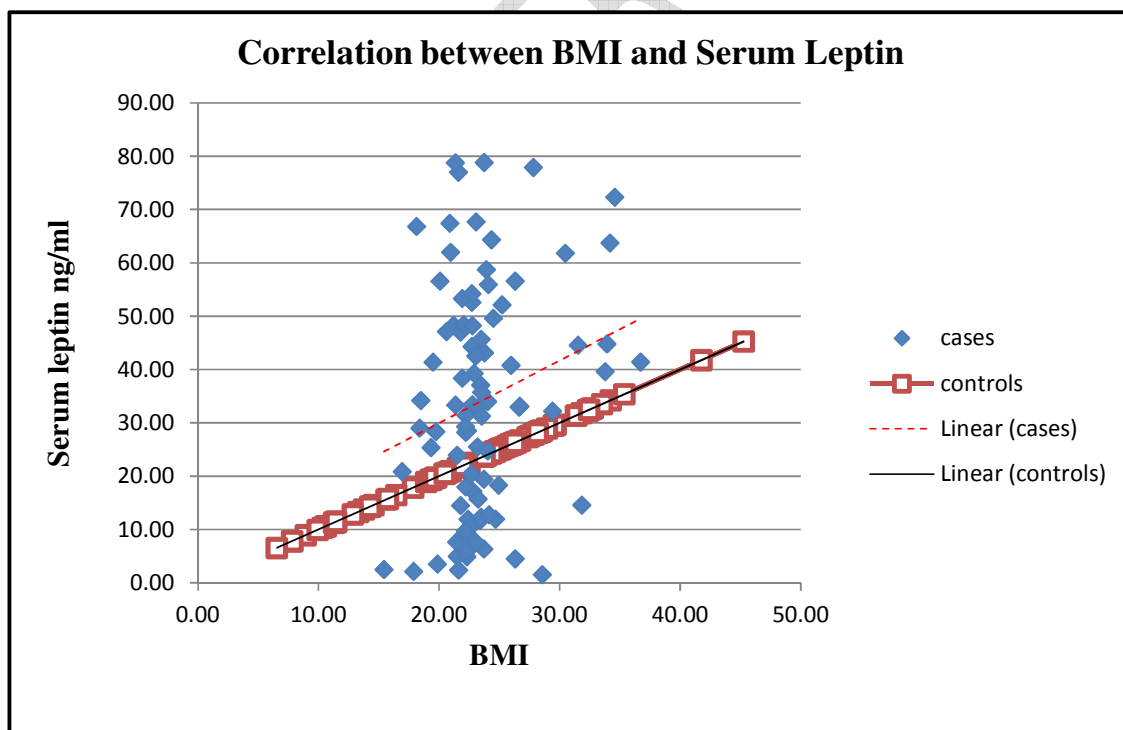
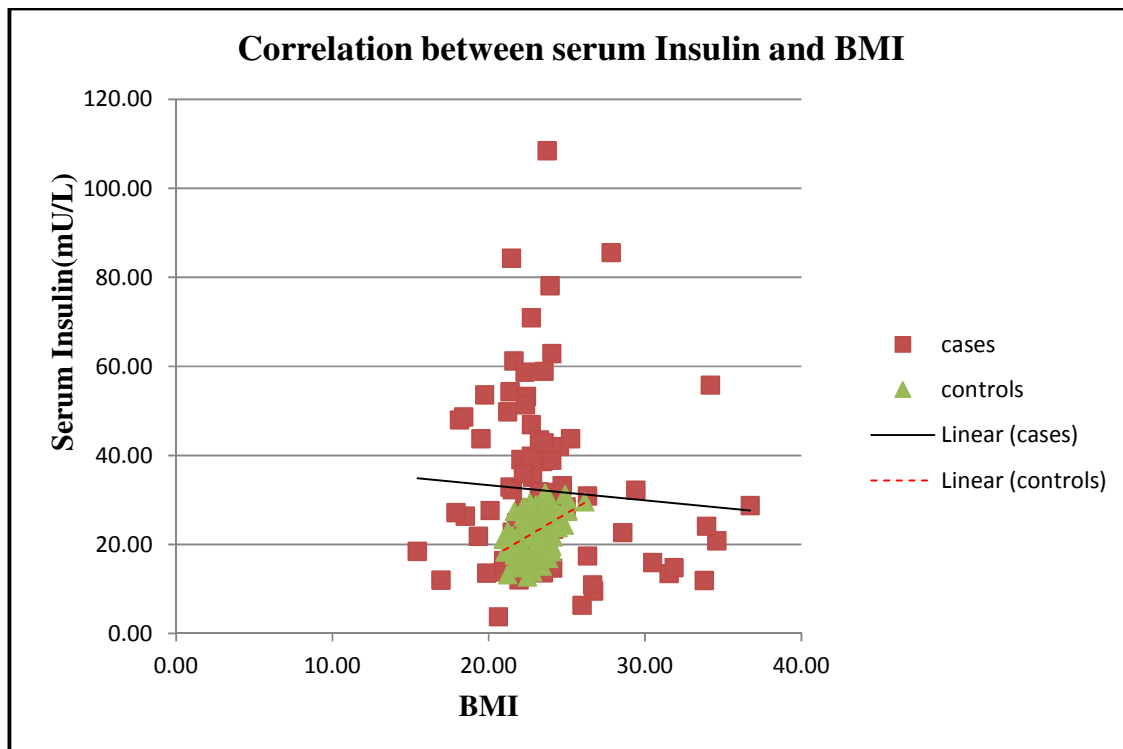


In the present study the mean leptin levels among cases and controls were comparable, but were not significant. The mean leptin level in cases was (34.14 ± 21.08) ng/ml and in controls was (23.38 ± 7.65) . Our study showed that leptin levels were raised in both cases and in healthy age matched women. Serum leptin levels were higher in PCOS cases when compared to controls. This is consistent with findings by T.E. Badawy et al and Al-Watify al, Mantzoros et al.^{8,9,10}

Graph3

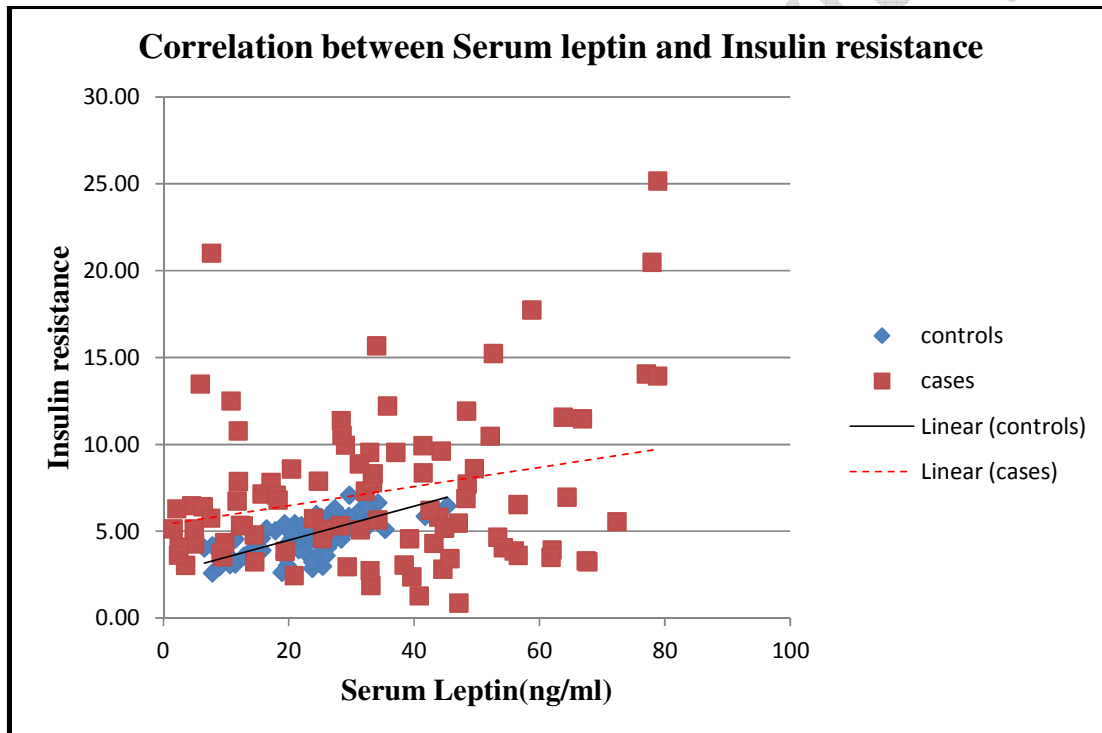


The Insulin resistance was calculated using HOMA-IR formula. Insulin resistance was compared between both groups. Insulin resistance was significantly raised in the cases group when compared with the control group ($P=.02$). The mean IR among the cases was (7.26 ± 4.4) and the mean IR among controls was (4.82 ± 1.07). The HOMA-IR value greater than 3.2-3.9 generally indicates insulin resistance. In our study mean HOMA-IR was (7.26 ± 4.4) in women with PCOS. This suggests increased occurrence of insulin resistance in PCOS. This is comparable with study results by Coviello et al¹¹ who reported higher HOMA-IR values in PCOS patients. Leptin levels correlated positively with BMI among the cases(group1) in our study($P=.03$), ($r=0.21$) and also among the controls (group2) in our study($P=.003$), ($r=1$). In our study PCOS women had higher mean BMI and leptin levels when compared to controls. Insulin levels correlated positively with BMI among the cases group1 in our study ($P=.004$) and ($r=0.21$) and this correlation was statistically significant. Among the controls group2 insulin levels correlated positively with BMI ($r=0.42$) and this correlation was not statistically significant ($P=.64$). Insulin levels were higher in women with BMI in the overweight and obese category. Although most of our cases were of lean weight and had BMI in the normal range high insulin and IR was detected in them showing impaired glucose metabolism.



Leptin levels correlated positively with IR among cases group1 ($r=0.25$) and this correlation was statistically significant ($P=.013$). Among the controls group2 this correlation was positive($r=0.6$) but not significant ($P=.80$). Leptin levels were higher in the cases group who

also had high IR when compared to healthy controls in our study. IR and hyperinsulinemia may also be factors that affect serum leptin levels as shown in studies on leptin and insulin and IR by Mohiti-Ardekani et al¹², Leroy P et al¹³. It had been shown that insulin directly induces leptin mRNA in adipocytes in vitro, suggesting that insulin may stimulate leptin secretion. The results of our study show higher leptin levels among the cases (group1) most of whom were in the lean body weight BMI range. This shows most of the PCOS patients were of lean weight and that fat cells in the abdomen were increased and had abdominal obesity. This is similar to findings by Joshi et al⁷ and Gill et al⁶ that PCOS women in india were more of lean weight and the increased insulin and IR caused the abdominal adipocytes to secrete more leptin.



Conclusion

A higher number of cases of PCOS that is around 66% were seen in young women in the age group 18 to 25 years. The majority of women diagnosed with PCOS were of lean weight and non-obese. In the present study hyperinsulinemia was noted in lean and obese PCOS cases.

In PCOS women the mean serum leptin levels and BMI was higher when compared to the control group.

Our study also showed higher serum insulin levels and IR among PCOS cases when compared to control group. Insulin levels were higher in women with BMI in overweight and obese category when compared to lean weight women.

References

1. Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. PCOS: Etiology, pathogenesis and diagnosis. *Nat Rev Endocrinol* 2011;7:219-31.
2. Laughlin G A, Morales AJ, Yen SSC. Serum leptin levels in women with polycystic ovary syndrome: the role of insulin resistance/hyperinsulinemia. *J Clin Endocrinol Metab* 1997;82:1692-6.
3. Barash IA, Cheung CC, Weigle DS, et al. Leptin is a metabolic signal to the reproductive system. *Endocrinology*.1996;137:3144-3147.
4. Brutis CA, Ashwood ER, Bruns DE. Diabetes mellitus. *Tietz textbook of clinical chemistry and molecular diagnostics*.5th ed. Elsevier;2012.p.1441-3.
5. Kandarakis D, Dunaif A. Insulin resistance and polycystic ovarian syndrome revisited: an update on mechanisms and implications. *Endocrinol Rev* 2012;33:981-1030.
6. Gill H, Tiwari P, Dabadghao P. Prevalence of PCOS in young women from North India: A community based study. *Indian J Endocrinol Metab* 2012;16:389-392.
7. Joshi B, Mukherji S, Patil A, Chauhan S et al. A cross sectional study of PCOS among adolescent and young girls in Mumbai, India. *Indian J Endocrinol Metab* 2014;18:317-324.
8. Badawy TE, El-Gharaib MN. Correlation between insulin, leptin and PCOS. *J of Basic and Clin Reprod Sc.*2014;vol 3:49-53.
9. Al-Watify. Abnormalities of hormone and inflammatory cytokines in PCOS women. *J Nat Sc Res.*2014;vol79:6-9.
10. Mantzoros CS, Bluher S. Leptin in reproduction. *Cur Op Endocrinol, Diabetes, Obesity.*2007;6:458-464.
11. Coviello S, Godarzi MO, Korenman SG. The importance of insulin resistance in PCOS. *Fertil Steril.* 2003;80:255-258.
12. Ardekani JM, Tarof N, Aflatonian A. Relationship between free leptin and insulin resistance in women with polycystic ovarian syndrome. *Iran J Reprod Med* 2009;7:53-8.

13. Leroy P, Dessolin S, Villageois P, Moon BC, et al. Expression of ob gene in adipose cells and regulation by insulin. J Biol Chem.1996;271:2365-2368.

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