



STUDY OF ASSOCIATION OF LEPTIN AND INSULIN RESISTANCE MARKERS IN PATIENTS OF PCOS

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ABSTRACT

Polycystic ovary syndrome (PCOS) the most common endocrine disorder, PCOS may present with hirsutism, acne, excessive androgen production, menstrual disturbances and subsequently, anovulation and infertility.

Leptin, a peptide hormone secreted from adipose tissue. Insulin resistance and hyperinsulinemia may also be factors that affect serum leptin levels. Aim of this study is to evaluate the association of leptin and insulin resistance markers in patients of PCOS.

SUBJECT AND METHOD: Sixty diagnosed cases of PCOS were enrolled in this study after informed written consent. The patients were diagnosed as PCOS as per Rotterdam criteria

All routine biochemical investigation were done. Insulin resistance was estimated using the homeostatic model assessment-insulin resistance (HOMA-IR) and HOMA-beta. HOMA-IR insulin levels and serum leptin levels was measured

RESULT: We found correlation between leptin and various insulin resistance markers although not significant

KEYWORDS :

INTRODUCTION

Polycystic ovary syndrome (PCOS) was first reported as Stein-Leventhal syndrome in 1935¹ and has since emerged as the most common endocrine disorder, affecting 18% of women in reproductive age group². PCOS may present with a constellation of clinical manifestations which include hirsutism, acne, excessive androgen production, menstrual disturbances and subsequently, anovulation and infertility³. The potential long-term consequences of PCOS include type 2 diabetes mellitus, hypertension, and cardiovascular disease.⁴

Obesity is a common occurrence in PCOS. There is ample evidence that overweight/obesity amplifies the clinical severity of PCOS and the risk of metabolic dysfunction like insulin resistance. The current 'epidemic' of obesity, therefore, has very serious implications on the long-term health of women with PCOS. However, it is unclear whether obesity is the cause or the effect of PCOS. Therefore, leptin the hormone product of the *ob* gene⁵ is currently the focus of research in these patients.

Leptin, a peptide hormone secreted from adipose tissue, is believed to carry signals from the adipocytes to the brain and thus has an important role in food consumption and energy balance⁶. Insulin resistance and hyperinsulinemia may also be factors that affect serum leptin levels as shown in some studies. It had been shown that insulin directly induces leptin mRNA in adipocytes in vitro, suggesting that insulin may stimulate leptin secretion⁷.

PCOS is a well-known state of chronic hyperinsulinemia due to a compensatory mechanism for insulin resistance, but a correlation between leptin concentration and hyperinsulinemia in patients with PCOS as an effect independent of adiposity has seldom been demonstrated⁸. Thus women with polycystic ovary syndrome (PCOS) serve as a model to assess the role of insulin resistance (IR) and chronic hyperinsulinemia on leptin levels.

Materials and Methods:

The study was conducted jointly by the department of Biochemistry and department of Obstetrics and Gynaecology, Lady Hardinge Medical College, New Delhi, India after Institutional ethical clearance. Sixty diagnosed cases of PCOS were enrolled in this study after informed written consent. The patients were diagnosed as PCOS as per Rotterdam criteria. Two out of three of the following are required to confirm the diagnosis: oligo- and/or anovulation; clinical and/or biochemical signs of hyperandrogenism; and polycystic ovaries (by ultrasound) Hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia, and androgen secreting tumors were excluded by specific laboratory analysis (cortisol,

ACTH, 17αOH-progesterone, and DHEAS).

A detailed history and clinical examination was done in all subjects in the study groups. Height and weight were recorded and body mass index (BMI) values were derived from Quetelet's formula (weight in kg/height in m²). Waist circumference was measured in a standing position midway between the lower costal margin and the iliac crest. Hip circumference was measured in a standing position at the maximum circumference over the buttocks.

Under aseptic conditions, 8 mL of fasting blood sample was drawn into plain and EDTA-NaF containing vacutainers from the antecubital vein. The vacutainer containing the blood samples were kept at room temperature for 30 min and were then centrifuged for the clear separation of serum or plasma. All routine biochemical assays were performed immediately after the serum or plasma was separated and rest serum samples were kept in -80°C.

The routine biochemical investigation includes fasting plasma glucose (FPG), liver function tests (total bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase), kidney function tests (urea, creatinine, uric acid), electrolytes (sodium, potassium, calcium, phosphate) and lipid profile (total cholesterol, triglyceride, high density lipoprotein (HDL). Routine biochemical parameters were estimated on same day on Beckmann Coulter synchron CX-9 fully automated analyser using standard reagents kits.

Insulin resistance was estimated using the homeostatic model assessment-insulin resistance (HOMA-IR) and HOMA-beta. HOMA-IR was calculated as the product of the fasting plasma insulin value (mU/ml) and the fasting plasma glucose value (mg/dl), divided by 405 (9). $HOMA \beta = 360 \times [Insulin] / ([Glucose] - 63) \% (Glucose \text{ in } mg/dl)^9$

Estimation of insulin levels were estimated by Chemiluminescence immunoassay (CLIA) in Access 2 Beckmann Coulter. Estimation of serum leptin levels was done by ELISA using leptin ELISA kit from (DRG.)

Statistical analysis

The data generated from this study was analyzed by using Statistical software SPSS version 13. The values are expressed as mean ± S.D with their 95 % confidence intervals. *p*-value < 0.5 was considered statistically significant. Pearson correlation and multiple linear regression analysis was performed to develop a regression model and to explore the predictive power of various insulin resistance markers in PCOS

RESULTS:

Table 1. Clinical features and demographic profile of study group (n=60)

Clinical features	Percentage(%)
Age≤19 yrs	10
Age≥19 yrs	90
Age of menarche at 11-14yrs	98
Overweight (BMI- 25-30)	23
Obesity (BMI ≥30)	30
Menstrual irregularities	96
Hyperandrogenic features	13
Infertility	50
Polycystic ovaries on ultrasound	100

Table 2.Serum leptin levels and insulin resistance markers in patient with PCOS(n=60)

parameters	Mean±S.D
FPG(mg/dl)	95.62±13.79
Fasting insulin(μU/ml)	20.44±13.84
HOMA-IR	5.42±5.59
HOMA-β	260.58±204.64
Leptin(ng/ml)	53.37±39.94
BMI(kg/mt ²)	27.1±5.8
WHR	0.8±0.07

Table3. Correlation of serum leptin and insulin resistance markers in patients with PCOS.(n=60)

parameters	Pearson correlation(r)	p-value
Fasting plasma glucose	0.036	0.784
Fasting insulin levels	0.159	0.225
HOMA-IR	0.148	0.258
HOMA-β	0.184	0.158
Body mass index	0.367	0.004*
WHR	-0.127	0.334

*p value <0.05 is considered significant.

Table 4.Multiple Linear Regression Analysis of HOMA -IR with Insulin Resistance Markers in PCOS (n=60)

Parameters	Coefficient of determination (r ²)	p-value
Fasting plasma glucose	.084	.025
Fasting insulin levels	0.876	0.0001*
HOMA -B	.417	<.0001*
Leptin	0.022	0.258
BMI	0.092	0.018
WHR	0.014	0.372

*p value <0.05 is considered significant.

HOMA-IR was taken as dependent variable
Multiple linear equation (y=a+b₁x₁+b₂x₂+b₃x₃+b₄x₄)

Table 5. Comparison of various insulin resistance markers between 2 groups according to BMI (≤ 25) and BMI > 25 values

Parameter	BMI ≤ 25 (n=28)	BMI > 25 (n=32)	p-value
	Mean ± SD	Mean ± SD	
Insulin (μU/ml)	17.97±9.70	22.61±16.50	0.198
GI-ratio	6.26±2.82	6.08±2.99	0.810
HOMA-IR	4.23±2.50	6.47±7.18	0.123
Leptin(ng/ml)	34.78±21.32	69.62±45.34	0.0001*
BMI(kg/m ²)	22.50±2.00	31.03±5.18	0.0001*
WHR	0.767±.054	.868±.053	0.0001*

*p value <0.05 is considered significant.

DISCUSSION

All 60 women completed the study. Polycystic ovary syndrome (PCOS) is an endocrine disorder characterized by menstrual disturbances, clinical and biochemical manifestations of hyperandrogenism and polycystic ovaries⁹.

The discovery of leptin, and its association with energy balance and the reproductive endocrine axis, has led to considerable interest in the association between polycystic ovary and leptin levels. Hence our study was planned to find the correlation between leptin levels and insulin resistance in women with PCOS.

HOMA-IR value greater than 3.2 to 3.9 generally indicates insulin resistance¹⁰. In our study HOMA-IR was 5.42±5.59 in women with PCOS. This suggests increased occurrence of insulin resistance in PCOS. Which is comparable with study results by Coviello et al¹¹ reported HOMA-IR value of 6.0±5.0 in patients with PCOS. We have also found that HOMA-IR is positively correlated with leptin but this correlation was not significant (r=0.148, p=0.258). Our results are similar to study conducted by Mancini et al¹² and Erturk et al¹³ who reported no significant correlation between leptin and HOMA-IR (r=0.317, p value>0.5).

Recent studies have shed some light on the possible relationship of insulin with leptin levels, Insulin has been shown to modulate leptin gene expression and increase in its levels, In our study we have observed a positive correlation between insulin and leptin levels, however it is not significant (r=0.159, p value 0.225). Our results are similar to the study by Erturk et al¹³ who reported that hyperleptinemia in women with PCOS is correlated with obesity and is not affected by insulin levels. However Remberg¹⁴ et al reported significant correlation (r=0.599, p=0.001) between insulin and leptin in PCOS. Our observations, along with recent studies suggest that alterations in endogenous insulin levels modulate leptin synthesis. In other words, obesity may mean that a young woman with polycystic ovary who would have otherwise been asymptomatic (or have had mild symptoms) will now suffer from hyperinsulinemia and insulin resistance.

In our study, leptin levels in women with PCOS were significantly correlated with BMI (r=0.367, p value 0.004). This result is comparable with study by Erturk et al¹³ who showed that BMI and leptin levels were significantly correlated (r=0.649, p value <0.0001). The result of our study also confirmed previously published data demonstrating a positive correlation between serum leptin and BMI in women with PCOS.

We found correlation between leptin and various insulin resistance markers although not significant. Since this is a cross-sectional study, we were only able to examine the leptin level and insulin resistance markers at one time-point. A greater effect of leptin on insulin resistance in PCOS may be found by studying the change in leptin levels over the time

Our study had a number of limitations. We evaluated only PCOS patients without a control group which might have helped in better analysis and ratification of our findings. We did not indulge in the evaluation of the molecular mechanisms (polymorphism studies, proteomics) for further validation of our observations. Financial and managerial constraints proved as limiting factors. Nonetheless, our study is a step forward in throwing light on the association of leptin with insulin resistance markers in women with PCOS especially in the Indian context.

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