ASSOCIATION BETWEEN LEPTIN AND ADIPONECTIN IN WOMEN WITH POLYCYSTIC OVARY SYNDROME IN NORTH INDIAN POPULATION

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ABSTRACT

Polycystic ovary syndrome (PCOS) is one of the most common endocrine abnormalities, characterized by biochemical hyperandrogenaemia, chronic anovulation, and polycystic ovaries. The objective of the present study to determine the association of adiponectin and leptin with women with PCOS in North Indian Women. In the hospital-based case-control study 100 women with PCOS and 100 controls were recuited from RAMA Medical College, Kanpur, India. Five ml venous blood sample was collected under aseptic precaution and transferred in the serum separator tubes. Leptin and adiponectin levels were assayed by human sensitive leptin double-antibody sandwich enzyme-linked immunosorbent one-step process assay (QAYEE-BIO life science) according to instructions provided with the kit. In the multivariable logistic regression analysis, leptin was found to be independently associated with 1.14 times higher risk for PCOS (OR 1.14, 95% CI 1.01 to 1.29). Receiver operating curve (ROC) analysis suggested excellent predictive accuracy of the multivariable model with Area under the curve 0.86. In the ROC analysis leptin was associated with 68% area under the curve for predicting PCOS using cut off value of >7.87, (P<0.001, Sensitivity 87.8, Specificity 49.4). Adiponectin level was not significantly associated with predicting PCOS (Area under the curve 0.54, P =0.30, Sensitivity 71.1, specificity 50.5, cut off value <10.2). To conclude we observed that higher leptin levels were independently associated with the risk of PCOS, however adiponectin level was not independently associated with the risk of PCOS.

KEYWORDS

Polycysitc Ovary Syndrome, Women, PCOS, Adiponectin, Leptin, North India

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine abnormalities, characterized by biochemical hyperandrogenaemia, chronic anovulation, and polycystic ovaries. Polycystic ovary syndrome (PCOS) was first described by Stein and Leventhal syndrome in 1935.[1] It is a heterogeneous collection of signs and symptoms with a mild presentation in some but a severe disturbance of reproduction and endocrine function. Its pathophysiology seems to be multifactorial and polygenic. The definition of PCOS is much debated, although it includes key salient features like the disturbed menstrual cycle, hyperandrogenism, and obesity. A simple blood-based biomarker may serve the purpose and improve the diagnostic accuracy for the PCOS. At present, there are no established blood-based biomarkers for the diagnosis of PCOS. Antimulerian hormone (AMH) is evolving as a promising biomarker for the diagnosis of PCOS as an additional biomarker along with ultrasound.[2] There is a clear need to discover reliable and readily available biomarkers for the diagnosis of PCOS. Leptin and adiponectin are protein hormones that play important role in the metabolic process could help us to diagnose PCOS. Leptin was the first fat cell-derived hormone (adipokine) for which gene is expressed in the hypothalamus a region of the brain that regulates the sensation of hunger and body weight. Adiponectin is a protein hormone that regulates the metabolic process like glucose regulation and fatty acid regulation. It is a protein hormone that is secreted from adipose tissue. This hormone is located in plasma in adequate quantity and is also being secreted from the placenta during the pregnancy period.

A meta-analysis involving a total of 19 studies with 991 women with PCOS and 898 controls showed that the leptin level was statistically significantly higher in PCOS subjects compared to controls (SMD 1.62, 95% CI 1.01 to 2.23).[3] Another, a meta-analysis which included 38 studies involving 1944 PCOS women and 1654 controls, demonstrated that statistically significantly low circulating adiponectin levels in women with PCOS (weighted mean difference -2.67, 95% CI -3.22 to -2.1).[4] However, heterogeneity was significantly higher across the studies included in these meta-analyses. Differences in the methodologies used, study design, and population-specific differences may explain the reason for heterogeneity observed in the meta-analyses. None of the studies included in this meta-analysis was from the Indian Population Well-designed studies are required to determine the precise estimate of exposure and outcome relationship and also the addition of studies will narrow the confidence interval for a precise estimate of the association of leptin and adiponectin level with PCOS. Therefore, we conducted a study aimed to determine the association of adiponectin and leptin with women with PCOS in North Indian Women.

MATERIALS AND METHODS

The study subjects were recruited in this hospital-based case-control study if they fulfilled the following inclusion and exclusion criteria. Inclusion criteria for cases were women diagnosed clinically as having PCOS, aged between 20 to 40 years, able to provide the written informed consent form, in good physical state, they were negative for serum Hepatitis B virus (HBV), Hepatitis C virus (HCV) and HIV. Cases were excluded if the women had any other reproductive disorder, were aged below 20 or above 40-years, had a known history or acquired thrombophilia or tumors in any part of the body. Inclusion criteria for controls were women aged from 20 to 40 years, normal fertile women without a history of PCOS, able to provide the written informed consent form in the good physical state, negative for serum HBV, HCV and HIV. Controls were excluded if females with any other reproductive disorder, females below 20 and above 40-year group, known history or acquired thrombophilia, known tumors in any part of the body. The study was approved by the Institutional Ethics Committee, approval was obtained from both cases and controls.

The convenience method of sampling was used for the selection of cases and controls in the present study. Data was collected in the standardized data collection form. Subjects for the present study were recruited from the RAMA medical college, Kanpur, India. Five ml venous blood sample was collected under aseptic precaution and transferred in the serum separator tubes. The serum was separated within an hour and stored at -20^{0}C until analysis. Leptin and adiponectin levels were assayed by human sensitive leptin double-antibody sandwich enzyme-linked immunosorbent one-step process assay (QAYEE-BIO Life Science) according to instructions provided with the kit.

The sample size was calculated to minimize type I & type II error. Assuming a minimum power of 80% and 5% significance level, the sample size was been calculated considering

the prevalence of PCOS at 10% and margin of error 5%, we calculated the sample size of 85. To control the loss of any sample and manual error the final sample size of 100 for each group was considered in the present study.

Data were collected and entered in MS Excel worksheets. Descriptive statistics were used to represent the baseline variables, number, and percentage for the categorical variables and mean and standard deviation for the continuous variables. The differences in the categorical variables were analyzed using a chi-square test. Differences in the continuous variables were analyzed using a student t-test if the distribution was normal. Central Limit Theorem was considered for meeting the statistical assumptions. Logistic regression analysis was done to assess predictors for PCOS. The confounding effects of covariates for which p value less then 0.10 in the univariable analsyis and having clinical relevance were adjusted in the multivariable logistic regression analysis. All the statistical analyses were conducted using licenced version of software STATA version 13.0

RESULTS

During the study period February, 2019 to February 2020 a total of 200 study subjects (100 patients attending OPD of Gynecology Department and 100 controls) were recruited from RAMA Medical College, Kanpur. We have also analysed the data as per LH and FSH ratio in which our statistical analysis demonstrated that LH: FSH ratio is statistically significantly higher in the women with PCOS as compared to controls (mean difference 0.67, Standard error 0.14, t = 4.49, P <0.001). PCOS women had statistically significantly raised blood cholesterol level as compared to controls (Mean difference = 7.78, Standard error 3.02, t = 2.57, P = 0.0109 (Table 1).

In the univariate analysis, we observed significantly higher level of serum leptin in women with PCOS (14.02±6.8) as compared to controls (9.86±5.9), P <0.001. Adiponectin level (10.2±5.9) was statistically significantly lower in the PCOS women compared to control (12.4±8.7) P =0.04. (Table 1). Leptin to adiponectin level was also significantly higher in cases as compared to controls (P =0.04)

Table 1: Comparison of demographics and risk factors between cases and controls

Variable	PCOS (Mean±SD)	Control (Mean±SD)	P value *
Age in years	27.07±4.4	26.2±3.1	0.11
LH	8.25±4.5	5.9±4.5	0.002
FSH	5.6±1.47	6.8±1.9	0.0001
TSH	5.01±4.07	3.68±2.25	0.015
Prolactin	13.8±7.5	12.6±4.4	0.27
Cholesterol mg/dl	175±15	167±22.2	0.01
Systolic blood pressure mm Hg	123.03±6.6	122.7±3.1	0.66
Diastolic blood pressure mm Hg	77.03±6.9	76.5±6.5	0.60
BMI (kg/m^2)	25.9±4.7	24.4±4.1	0.01
Leptin	14.02±6.8	9.86±5.9	<0.001
Leptin for BMI>25	16.2±6.6	11.9±6.2	0.0009
Leptin for BMI≤25	9.12±4.20	7.5±4.5	0.14
Adiponectin	10.2±5.9	12.4±8.7	0.04
Adiponectin for BMI>25	7.7±3.7	9.3±6	0.10
Adiponectin for BMI≤25	15.1±6.5	15.7±9.8	0.79
Leptin/Adiponectin ratio	2.03±1.79	1.45±1.75	0.03
Leptin/Adiponectin ratio BMI>25	2.5±1.8	1.9±2.08	0.14
Leptin/Adiponectin ratio BMI<25	0.83±0.58	0.95±1.16	0.63

***independent sample t test used to compare mean difference between two groups**

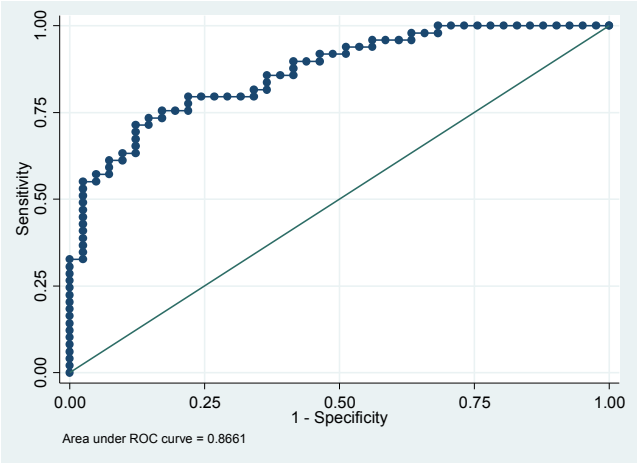

Fig. 1: Predictive accuracy of multivariable logistic regression model

Variables included in the multivariable analysis (FSH, LH, Cholesterol, BMI, Lepin and Adiponectin suggested leptin)

(Table 1). In the multivariate logistic regression analysis with variables FSH, LH, Cholesterol, BMI, Lepin and Adiponectin suggested that leptin levels independently associated with 1.14 times higher risk for PCOS (OR 1.14, 95% CI 1.01 to 1.29) (Table 2). Receiver operating curve (ROC) analysis suggested excellent predictive accuracy of multivariable model with Area under the curve 0.86. In the ROC analysis leptin was associated with 68% area under the curve for predicting PCOS using cut off value of >7.87, (P<0.001, Sensitivity 87.8, Specificity 49.4) (Fig. 2). Adiponectin level was not significantly associated with predicting PCOS (Area under the curve 0.54, P =0.30, Sensitivity 71.1, specificity 50.5, cut off value <10.2). (Fig. 2). In the subgroup analysis for obesity vs normal, we noted statistically significantly higher leptin level and statistically significantly lower adiponectin level among obese persons across all groups (Overall study

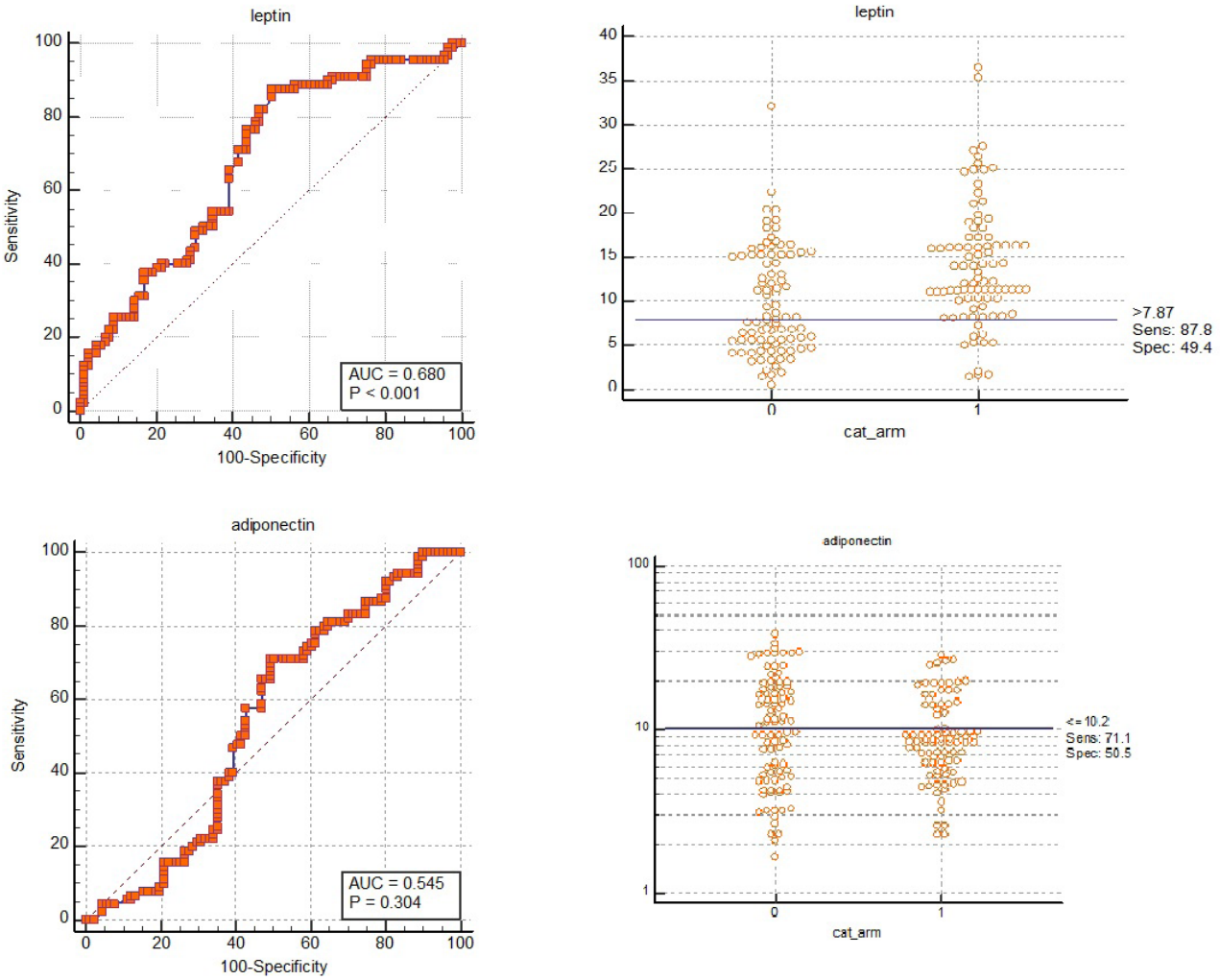

Fig. 2: Receiver operating characteristics curves for leptin and adiponectin level

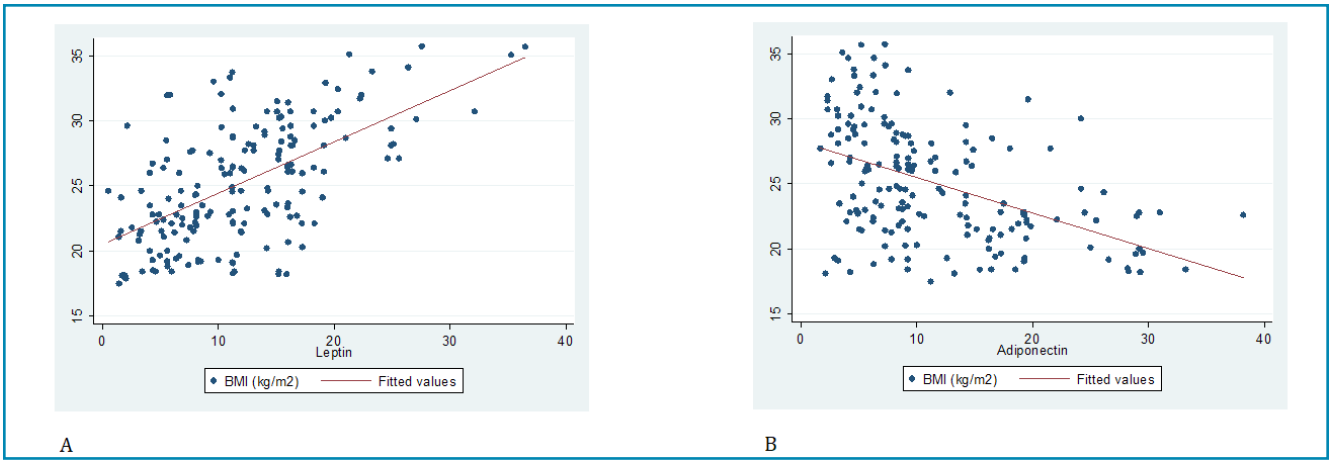

Fig. 3: Scatter plots for correlation of leptin and adiponectin with BMI

Table 2 : Multivariable logistic regression analysis : Dependent variable : PCOS/Control

Variable	Odds Ratio	Std. Err.	z	P>z	[95% Conf.	Interval]
FSH	.7066771	.1375344	-1.78	0.074	.4825686	1.034863
LH	1.915689	.4007506	3.11	0.002	1.271331	2.88663
Cholesterol	1.040725	.0170023	2.44	0.015	1.007929	1.074588
BMI	.8898053	.0765471	-1.36	0.175	.751742	1.053225
Leptin	1.147003	.0708055	2.22	0.026	1.016294	1.294524
Adiponectin	.9430213	.0434028	-1.27	0.202	.8616775	1.032044

Abbreviation : FSH, Follicular Stimulating Hormone, LH, Lutenizing hormone, BMI, Body mass index

Table 3: Stratified analysis as per BMI for comparison of lepin and adiponectin between cases and controls

	Obese	Normal	P value
Leptin			
Overall study subject	14.3±6.8	8.17±4.4	<0.001
PCOS	16.2±6.6	9.1±4.2	<0.001
Control	11.9±6.2	7.5±4.5	0.003
Adiponectin			
Overall study subject	8.4±4.9	15.4±8.6	<0.001
PCOS	7.7±3.7	15.1±6.5	<0.001
Control	9.3±6	15.7±9.8	0.0003

subjects, PCOS, Controls) (Table 3). A positive correlation was seen between leptin level and BMI which indicated a significant increase in leptin level with an increase in BMI (Fig. 3a). A negative correlation was seen between adiponectin level and BMI which indicated a significant decrease in adiponectin level with an increase in BMI (Fig. 3b).

DISCUSSION

The present study observed that high leptin levles acts as an independent predictor for the diagnosis of PCOS, however adiponetin levels did not turn as independent predictor for PCOS. With obesity, leptin was positively associated and adiponectin was negatively associated with degree of obesity in women with PCOS.

Our findings in women with PCOS is consistent with earlier studies reported in women with PCOS, demonstrating that obesity primarily determines serum leptin and adiponectin level.[5,6]

Leptin is mainly produced by the adipocytes and considered as a polypeptide hormone for the regulation of normal body weight. Several studies have observed a strong association of circulating leptin with obesity, which has also been associated with PCOS, a major form of diovulatory infertility in women. It is important in regulating energy homeostasis and has an impact on the reproductive systems in diverse ways. PCOS, the common diovulatory infertility, is considered by chronic anovulation, hyperandrogenaemia, insulin resistance, and a high incidence of obesity; therefore, leptin may be related to pathogenesis of PCOS. A study concluded that further studies are required to elucidate the independent correlations between serum leptin levels and other biochemical and phenotypic features of PCOS in these women.[7] Thus leptin level in women with PCOS women emerged as useful tool to assess ovarian function. Several studies have shown statistically significantly higher level of serum leptin in women with PCOS,[8–10] however, some of studies failed to derive such an association.[11,12] A meta-analysis reported in 2016 which included a total of 19 studies, observed that leptin levels statistically significantly higher women with PCOS compared to controls (SMD, 1.62, 95% CI 1.01 to 2.23). Substantial heterogeneity was noted between the studies included in this meta-analysis. The variability in insulin resistance, BMI status and location of study may explain the heterogeneity observed in this meta-analysis. In the subgroup analysis this study demonstrated no significant association of leptin level and PCOS in women with BMI<25, however statistically significantly higher leptin level was observed when analysis was restricted in PCOS women with BMI>25.[3] Our study also observed the similar association which noted statistically significant association in the analysis restricted to BMI>25. In the subgroup of BMI≤25 moderately higher level of leptin in non-obese cases but the differences was not statistically significant. Our study findings also showed the positive linear correlation of leptin levels with BMI. Interestingly, our multivariate analysis demonstrated that independent association of leptin levels with risk of PCOS even after adjustment of confounding effect caused due to BMI.

The fat tissue hormone adiponectin has been emerged as a significant biomarker in the pathogenesis of PCOS. A study reported by Panidis *et al.* observed that significantly lower adiponectin levels in the obese PCOS subjects in comparison to normal weight PCOS subjects however, no statistically significant difference was noted in adiponectin levels in women with normal weight PCOS women compared to normal-weight women without PCOS.[13] The findings of this study indicated a strong inverse relationship between adiponectin levels and obesity. Preclinical studies have shown that adiponectin levels might exert an advantageous role in raising insulin sensitivity and antidiabetic effect. Remarkable insulin resistance along with obesity has been noted in women with PCOS. Many studies in the literature have shown that lower levels of adiponectin in women with PCOS were associated with a negative association with insulin resistance and a positive association with obesity.[14–16] On the other hand, some studies did not observe any statistically significant association between adiponectin levels and PCOS.[13,17,18] Few studies have reported an association of adiponectin level with PCOS that was independent of BMI and severity of insulin resistance.[19,20] The findings of the present study did not show an independent association between adiponectin levels and PCOS. Thus it appeared that the lower serum adiponectin levels were more likely to be attributed to obesity rather than PCOS. The adipose tissue hormone adiponectin has been playing a major role in the pathogenesis of PCOS. Adiponectin has antiatherogenic,anti-diabetic, anti-inflammatory, and insulin-sensitizing effects, and is inversely related to the degree of adiposity in healthy individuals.

Limitation: Small sample size study limited the power to generalize the study results. The insulin resistance level was not measured which may have an influential effect on exposure and outcome relationship.

Conclusion: The findings of the present study demonstrated that higher leptin levels were independently associated with the risk of PCOS, however adiponectin level was not independently associated with the risk of PCOS. As expected, a linear positive relationship between leptin levels and BMI was observed. Elevated leptin levels may contribute to the etiology of PCOS, but further, well-designed prospective studies are still needed to elucidate the precise association between leptin level and PCOS.

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Conflict of Interest: None

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