British Journal of Medicine & Medical Research
18(5): 1-9, 2016, Article no.BJMMR.29265
ISSN: 2231-0614, NLM ID: 101570965

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The Intricate Relationship between Insulin Resistance and Adipokines in Non-obese Patients with Polycystic Ovarian Syndrome

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AD, SR, NC and AB designed the study. Authors SR and AD wrote the protocol and wrote the first draft of the manuscript. Authors AD, SR and RM managed the literature searches and analyses of the study performed the spectroscopy analysis. Authors SR, AD and RM managed the experimental process. Authors NC and AB selected the patients. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/29265
Editor(s):
(1) Menelaos Zafrakas, Professor of Obstetrics and Gynecology, Alexander Technological Educational Institute of Thessaloniki, Thessaloniki, Greece.
Reviewers:
(1) O. Wai Sum, University of Hong Kong, Hong Kong.
(2) Waleed S. Mohamed, Taif College of Medicine, Egypt.
(3) Nadeem Afzal, University of Health Sciences, Lahore, Pakistan.
(4) Ione Maria Ribeiro Soares Lopes, Federal University of Piaui, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/16632

Opinion Article

Received 31st August 2016
Accepted 10th October 2016
Published 22nd October 2016

ABSTRACT

Background: Dyslipidemia and metabolic complications related to Insulin resistance are closely linked to patients suffering from polycystic ovarian syndrome (PCOS).
Aims: To find out the changes in serum testosterone, leptin and adiponectin levels and explore their relative importance in alterations of insulin resistance (IR) in non obese PCOS patients.
Study Design: Hospital based, case control, non interventional study. Both cases and control subjects were selected following the method of convenience according to prefixed inclusion and exclusion criteria.
Methodology: In 33 non obese PCOS patients and 35 controls Leptin, insulin, testosterone, leutinizing hormone (LH), follicular stimulating hormone (FSH) and adiponectin were measured by

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enzyme linked immunoassay (ELISA). Fasting plasma glucose (FPG) was measured by spectrophotometric method. Insulin resistance (IR) was calculated through homeostatic model for assessment of insulin resistance (HOMA-IR) technique. Waist hip ratio (WHR) and body mass index (BMI) were calculated as anthropometric parameters to assess distribution of body fat.

Results: Mean +/- Standard error of mean values for the HOMA-IR (*1.98 +/- 0.19 vs 1.04 +/ - 0.04*), FBG *(4.98 +/- 0.27 vs 4.52 +/- 0.07)* serum leptin (*12.55 +/- 1.00 vs 8.23 +/- 1.03*), serum testosterone (*1.19 +/- 0.07 vs 0.41 +/- 0.04*), serum LH (*30.74 +/- 1.73 vs 5.69 +/- 0.44*), serum FSH (*10.93 +/- 0.38 vs 6.29 +/- 0.46*) and WHR (*0.82 +/- 0.01 vs 0.74 +/- 0.01*) were significantly elevated in the PCOS patients compared to the control group. On the other hand, serum adiponectin was significantly decreased in PCOS group (*11.41 +/- 1.21 vs 23.55 +/- 2.94*) while BMI showed no significant difference. Although, individual bivariate correlation analysis suggested IR to be significantly associated with serum leptin, adiponectin, testosterone and WHR, but multivariate linear regression analysis revealed significant predictive values for serum leptin (β = *.467*,), adiponectin (β = *-.324*,) and testosterone (β = *.266*,) only on the HOMA-IR.

Conclusion: Increased androgen and leptin along with decreased adiponectin levels have crucial determining effects on increased IR that might play a major role in mediating the pathogenesis and metabolic abnormalities in non obese PCOS.

Keywords: Polycystic ovarian syndrome (PCOS); insulin resistance; leptin; adiponectin; non obesity; testosterone; LH:FSH ratio.

ABBREVIATIONS

BMI: Body mass index; FPG: Fasting plasma glucose; HOMA-IR: Homoeostatic model for assessment of insulin resistance; IR: Insulin resistance; MS: Metabolic syndrome; PCOS: Polycystic ovarian syndrome; WHR: Waist hip ratio.

1. INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a heterogenous group of fertility disorder that affects almost 6-12% of females in their reproductive age group, [1] its prevalence depending on different ethnic, genetic and environmental factors. The disease generally consists of amenorrhea, excess androgens, chronic anovulation, polycystic ovary and reduced fertility [2]. At least two of the following criteria must be present for PCOS to be diagnosed in a woman: Oligomenorrhoea, hyperandrogenism and polycystic appearance of ovary on transvaginal ultrasonography [3]. In addition to the reproductive abnormalities, several metabolic derangements like increased insulin resistance, deranged body fat metabolism and premature atherosclerosis increase the overall morbidity of the disease significantly [4,5]. The chief mediators of these complications have been the confounding factors like dyslipedemia, obesity and type 2 diabetes mellitus that increase the risk of metabolic syndrome and cardiovascular mortalities significantly in these patients [6-8].

Body weight in humans is maintained by a complex interplay between dietary behavior, abundance of food supply, locomotive and reproductive activities and several other environmental factors. Recent advances have suggested that adipokines play not only an important role in maintaining body weight but a much more integrative role in the overall metabolic aspects in humans. They are supposed to play major regulatory roles in enzyme catalyzed reactions, hormone secretion, homeostasis of glucose metabolism, regulation of gene expression, and nutrient flux to equilibrate under different conditions of metabolic changes. Leptin, a 16 kDa molecule is secreted from adipocytes and play a major role in relaying the energy balance to the feeding centre of the brain. In addition, it is found to play an important regulatory role in reproduction, locomotion, angiogenesis and blood pressure [9]. Leptin has been found to be secreted by granulosa cells also and act on the thecal and granulosa cells themselves through its cognate receptors. In PCOS, this paracrine function of leptin becomes particularly important in development of insulin resistance [10]. In PCOS patients, leptin levels have been described to correlate well with insulin resistance indicator HOMA-IR [11]. Adiponectin is another adipokine secreted exclusively from the adipocytes and is negatively correlated with the insulin resistance, obesity and dyslipidemia [12,13]. A high value of leptin and low value of adiponectin have been reported to be associated

with increased prevalence of metabolic syndrome in PCOS [14]. In addition, insulin resistance has been described as a major predictor for hyperandrogenemia and abdominal obesity that precipitate an early cardiovascular deaths in these patients [15].

Recent studies have indicated an increasing trend of PCOS affecting the non obese females in their reproductive age group. However, even in non obese females, insulin resistance is reported to be significantly associated with abdominal fat and hyperandrogenemia [15]. Studies have indicated that insulin resistance in non obese PCOS is mainly secondary to a reduced intrinsic insulin sensitivity due to defective insulin receptor and deranged post receptor signal transduction cascade for insulin signaling pathway along with reduced cellular uptake of blood glucose [16]. Therefore, there are conflicting results and lack of congruency regarding the effect of obesity on the pathogenesis of insulin resistance in PCOS [17-19]. Although, levels of serum leptin have been found to correlate directly with the degree of obesity in general, studies have showed raised serum leptin levels in PCOS patients independent of BMI, WHR and waist circumference [20] that might reflect an independent leptin resistance inherent to this syndrome.

Keeping these factors in mind, we hypothesized that there might be an intricate linkage between insulin resistance and adipokine resistance in non obese PCOS that may further complicate their metabolic status. Accordingly, the present study was carried out to test its outcome in non obese PCOS patients in our region.

2. MATERIALS AND METHODS

The study was conducted in the Department of Biochemistry & Department of Gynecology and Obstetrics of a tertiary care Medical College and Hospital of Eastern India during the period of one year from April 2015 to April 2016.

2.1 Selection of Study Subjects

Cases were selected from the patients attending Dept. of Gynecology on the basis of convenience method according to the following inclusion and exclusion criteria as mentioned below:

2.1.1 Inclusion criteria

i) Age: 15–55 years (Mean age being 35 yrs with an SEM of 0.85).

ii) PCOS diagnosed by Rotterdam criteria 2003 criteria for selection of the patients as 2 out of 3 criteria:

a) oligo- and/or amenorhhoea as evident from patients menstrual history.
b) clinical and/or biochemical signs of hyperandrogenism as evident from LH, FSH and testosterone levels.
c) polycystic ovaries as evident from ultrasonography.

2.1.2 Exclusion criteria

i) Patients with hyperprolactinemia, Cushing syndrome, acromegaly, hypothyroidism, ovarian failure, any other endocrinological disorder and simple obesity were excluded based on history, clinical examination, anthropometric measurements and appropriate laboratory tests wherever required.

a) Patients with any type of bacterial, viral or fungal infection.
b) Patients with any chronic disorder like renal diseases, hypertension or any malignant diseases.
c) Other endocrinological etiologies such as congenital adrenal hyperplasia, Cushing syndrome and androgen-secreting tumors as evident from ultrasonography.

Following the above inclusion and exclusion criteria 33 cases and 35 age and BMI matched control subjects were selected for the present study for analysis of required biochemical parameters. Control subjects were selected from the normal healthy women accompanying the patients with a mean age of 33 years (SEM of 0.81). First degree relatives were excluded from selection of controls to avoid any genetic susceptibility.

2.2 Measurement of Study Parameters

Fasting venous blood sample was obtained after 12 hr fasting maintaining strict aseptic protocol and study parameters were measured in 33 case and 35 control as follows:

i) Serum Insulin level was measured by streptavidin coated sandwich ELISA method from the reagent kit obtained from Accubind, USA.
ii) Fasting blood glucose (FBG) in the plasma was measured by standard

spectrophotometric method using GOD-POD reagent obtained from Erba diagnostics, Germany.

iii) Serum levels of Leptin, Adiponectin and Testosterone were measured by strepatavidin coated sandwich ELISA from Accubind, USA.

iv) Measurement of HOMA-IR, the marker of insulin resistance: Although, hyperinsulinemic euglycemic clamp technique is the gold standard for measuring insulin resistance, it is time consuming, expensive, invasive and requires close monitoring under admission to combat any accidental hypoglycemia. Compared to it homeostatic model assessment of insulin resistance (HOMA-IR) is much safer and simpler method for assessing insulin resistance under a stable fasting blood glucose and insulin level [21]. In the present study, we adopted HOMA-IR for assessing the insulin resistance. It was calculated as: fasting insulin (µu/ml)×fasting glucose (mmol/lit)/22.5.

2.2.1 Quality control for the test procedures

All test procedures were prevalidated reagent kits. For all study parameters the coefficient of variation (CV) was maintained lower than 10 percent.

2.3 Statistical Analysis

The present study was conducted in 33 PCOS cases and 35 healthy age matched controls. All data were checked for normal distribution by graphical analyses like box plot and histogram along with statistical analyses like Kolmogrov-Smirnov test. On finding their distribution as near normal, group statistics and independent t test were performed for assessing the difference between their mean values. Strength of association between different study parameters were analyzed by bivariate Pearson's correlation study. Relative importance of predictive values of adipokines, body weight markers and androgen level on HOMA-IR was assessed by multiple linear regression analysis. All statistical analyses were performed by using SPSS software Version 17.0 obtained from IBM, USA. A *P* value of less than 0.05 was considered as statistically significant for maintaining a 95% confidence interval.

3. RESULTS

Results from the graphical outputs and a *P* value greater than 0.05 in the Kolmogrov-Smirnov test pointed towards a near normal distribution of the data (Figs. 1a and 1b).

A mean BMI of 24.8 signified our PCOS patients to be non obese with no significant difference with that of the normal healthy controls (mean BMI = 23.4, *P* = 0.064, Table 1). From the mean values and their standard errors shown in the Table 1, it was evident that significantly high levels of serum LH, FSH and testosterone with a higher ratio of LH:FSH in the PCOS patients (about 3:1) show the characteristic biochemical criteria of the disease in our case group. Simultaneously higher levels of serum leptin and the HOMA-IR along with a lowered adiponectin level ($P < 0.05$ for all) in the case group cued towards an elevated IR that is associated with high serum leptin and low adiponenctin levels in our non obese PCOS patients.

Table 1. Group statistics for the study parameters in cases (N = 33) and controls (N = 35)

	Case (Mean /SE)	Control (Mean /SE)	T	*P*
Leptin in pg/ml	12.55/1.00	8.23/1.03	2.83	*.007**
Adiponectin in pg/ml	11.41/1.21	23.55/2.94	-4.4	*< .001**
HOMA-IR	1.98/0.19	1.04/0.04	3.3	*.002**
FBG (mmol/L)	4.98/0.27	4.52/0.07	1.31	*.199*
BMI	24.89/0.56	23.41/0.36	1.89	*.064*
WHR	0.82/0.01	0.74/0.01	3.9	*< .001**
Testosterone in ng/ml	1.19/0.07	0.41/0.04	7.3	*< .001**
LH in mIU/ml	30.74/1.73	5.69/0.44	11.05	*< .001**
FSH in mIU/ml	10.93/0.38	6.29/0.46	7.54	*< .001**

**P value considered to be significant at P < 0.05 for 95% confidence interval*

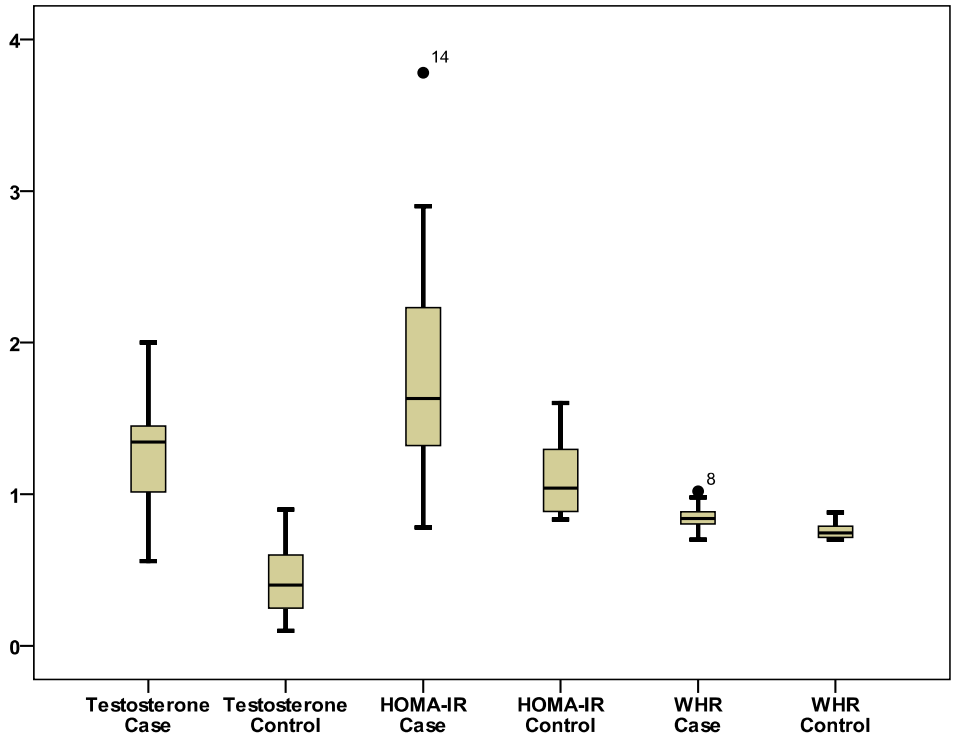

Fig. 1a. Box plot to show the distribution of individual study parameters in both cases and control subjects

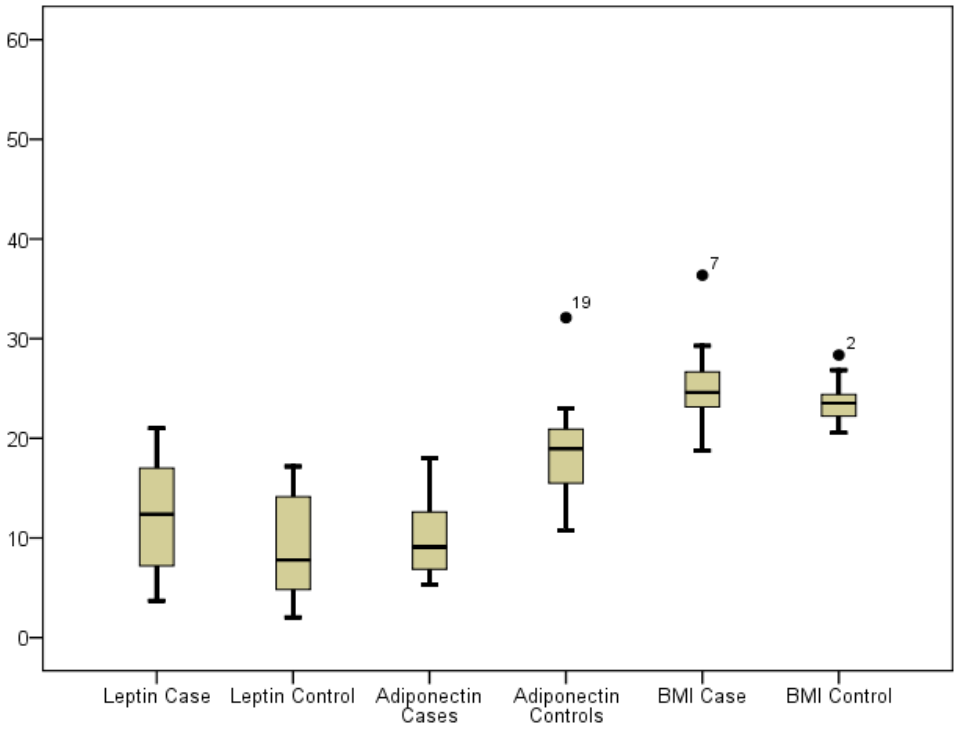

Fig. 1b. Box plot to show the distribution of individual study parameters in both cases and control subjects

Table 2. Pearson's bivariate correlation analysis for showing the strength of association between study variables in the case group (N = 33)

		BMI	WHR	Serum adiponectin	Serum leptin	HOMA-IR	Serum testosterone
BMI	R	1	.360	.333	-.052	-.022	.030
	P		.040*	.072	.785	.907	.868
WHR	R	.360	1	-.241	.617	.414	.184
	P	.040*		.199	< .001*	.023*	.305
Serum adiponectin	R	.333	-.241	1	-.389	-.603	-.372
	P	.072	.199		.033*	.001*	.043*
Serum leptin	R	-.052	.617	-.389	1	.693	.394
	P	.785	< .001*	.033*		< .001*	.031*
HOMA-IR	R	-.022	.414	-.603	.693	1	.569
	P	.907	.023*	< .001*	< .001*		.001*
Serum testosterone	R	.030	.184	-.372*	.394*	.569	1
	P	.868	.305	.043	.031	.001*	

** Correlation is significant at the 0.05 level (2-tailed).*
R: Pearson's bivariate correlation coefficient.
**P value considered to be significant at P < 0.05 for 95% confidence interval*

This was more conclusive from the results of bivariate correlation analysis shown in the Table 2, where serum leptin and adiponectin showed a direct and inverse correlation respectively with the HOMA-IR (correlation coefficient = 0.693 and -0.603 respectively and *P < 0.001* for both). The HOMA-IR showed a significant positive correlation with WHR (correlation coefficient = 0.414, *P = 0.023*) without any such association with the BMI (correlation coefficient = -0.022, *P = 0.907*) suggesting that IR in the PCOS patients is more significantly related with the abdominal or visceral obesity in comparison to the overall body fat distribution.

With HOMA-IR emerging out as a major determinant for metabolic abnormalities noted in PCOS, we proceeded to analyze its dependence on other important parameters in the present study by performing the multivariate linear regression assay. From its results as shown in the Table 3, it was evident that although WHR showed an independent direct association with insulin resistance in the bivariate correlation study (Table 2) it did not show a significant predictive value on the insulin resistance among the case group when considered together with other variables like leptin, adiponectin and testosterone. Rather, raised levels of testosterone and leptin showed positive predictive values for increased HOMA-IR (β = 0.467, *P = 0.007* and 0.266, *P = 0.049* respectively), whereas, lowered levels of serum adiponectin predicted an elevated insulin resistance (β = -0.324, *P = 0.019*).

Table 3. Multiple regression analysis to find out the dependence of insulin resistance on other study variables in PCOS patients (N = 33)

Model		Standardized Regression Coefficients Beta	*P value*
1	(Constant)		*.535*
	Serum leptin	.467	*.007**
	Serum adiponectin	-.324	*.019**
	Serum testosterone	.266	*.049**
	WHR	-.008	*.955*

Dependent variable: HOMA-IR
**P value significant for 95% confidence interval*

4. DISCUSSION

Higher values of leptin and insulin with lower values of adiponectin as found in our study group merit special mention as they are potent predictors for cardiovascular complications in PCOS even without obesity, particularly in the context of metabolic syndrome whose prevalence is much higher in the PCOS patients (8.2 to 14.3%) compared to the normal age matched women (2.7 to 6.6) [22]. Adiponectin is a 30 kD molecule secreted only from adipocytes. It has a significant potential role on insulin sensitivity and decreased adiponectin levels have been found to be associated with increased insulin resistance, dyslipedemia and endothelial complications in most cases [13,23]. Although, it

has been found to correlate negatively with obesity but the relative importance of its association with general and abdominal obesity in PCOS patients has not been studied adequately so far. Our study notably showed a definite association (Table 2) along with a positive predictive effect (Table 3) of adiponectin with lowering the insulin resistance in PCOS patients even in absence of obesity. Insulin resistance along with the accompanied hyperinsulinemia is itself a major link between PCOS and MS. Some studies have reported a high prevalence of insulin resistance as much as 32% in PCOS patients [24]. Although hyperandrogenism is a classical defining feature of PCOS that potentiates the insulin resistance, linkage of insulin resistance with cardiovascular complications in PCOS is more prominent. Insulin resistance in PCOS potentiates the hyperandrogenemia induced infertility by reducing the hepatic synthesis of sex hormone binding globulin [25], early follicular growth by reducing the synthesis of insulin like growth factor I (IGF-1) binding protein [26] and increased LH secretion [27]. Importantly, in the present study, WHR, the marker of visceral obesity, did not show a significant predictive value on the insulin resistance in PCOS patients when considered with other covariates like testosterone, leptin and adiponectin. Although, it individually exhibited a direct correlation with the HOMA-IR in bivariate correlation analysis, the lack of its predictive value on HOMA-IR in the multivariate study indicates that hyperandrogenemia, increased leptin levels and decreased adiponectin values are more important predictors in comparison to abdominal obesity for increasing the insulin resistance in the non obese PCOS patients. Insulin resistance in PCOS is not always linked to obesity exclusively as several studies reported insulin resistance in non obese PCOS patients [28]. However, increased body weight in PCOS potentiates hyperandrogenemia, oligomenorrhoea and metabolic complications and several obesity related genes and hormones (adipokines) were found to be associated with PCOS [29]. Although, obesity is attributed to generate a state of insulin resistance and hyperinsulinemia leading to androgen excess and high LH levels, resistance to both insulin and leptin occur in part, at the level of hypothalamus which is independent of obesity. This may explain a raised insulin and leptin levels with lowered adiponectin values in our non obese PCOS patients in the present study.

Leptin, as per se, functions as an appetite reducer by acting on the feeding center of hypothalamus and helps in keeping the body weight constant by regulating food uptake. But in obese people as well as in the PCOS obese women leptin resistance plays a significant role in inducing and maintaining the increased fat store in the body. Insulin resistance and hyperandrogenemia are another two confounding factors for increased fat deposition particularly the increased prevalence of abdominal obesity observed in PCOS women [15]. Although, the relationship between an increased leptin levels and PCOS is not clear till now, still increased leptin resistance in these women have been reported to be associated with increased prevalence of MS in them. Leptin and insulin both interact with each other at the level hypothalamus-pituitary-ovarian axis through receptor cross talk as both act via tyrosine kinase mediated signal transducer and activator of transcription (STAT) proteins. Hence, resistance to either insulin and leptin leads a deranged gonadotrophin release from the hypothalamus resulting in subfertility or infertility. However, only individual resistance of either of these hormones at the hypothalamic level are reported to produce relatively only subtle PCOS phenotypes. The synergy between the insulin and leptin in regulating the reproductive physiology is underscored by the observation that knockout mutation of the common receptor 2 (Irs2) for both insulin and leptin, rather than the insulin receptor 1 (Irs1) exclusively for insulin, produces metabolic, reproductive and ovarian features of PCOS in experimental rats in addition to abnormal glucose tolerance [30]. In our study we furthermore report increased levels of both leptin and HOMA-IR in non obese PCOS females. Although, metabolic abnormalities have been described to be more prominent in obese PCOS, but some recent studies have reported higher levels of fasting blood glucose and dihydroepiandrosterone (DHEA) in non obese PCOS in comparison to the greater potential effect of hepatoportal glucose metabolism [15]. A strong correlation of leptin and adiponcetin with WHR without any such relationship with the BMI (Table 2) strengthens the metabolic link of adipokines with visceral fat deposition more importantly than the generalized body fat deposition in these patients. All these observations, along with our study findings suggest that the genetic susceptibility and environmental factors play major roles in the pathogenesis and biochemical abnormalities of

PCOS with obesity acting as an independent confounding risk factor.

5. CONCLUSION

In conclusion, results of the present study suggest that obesity may not be a major contributing factor for generating metabolic abnormalities characteristics of PCOS as previously thought, particularly in the context of variation in ethnicity, environment and genetic setup. Rather, several other causative factors like insulin resistance, leptin resistance, lowered adipokine values play more important roles in some circumstances in mediating the metabolic complications of the disease. The exact mechanism of it is still not elucidated and needs further exploration regarding the ethnic, genetic and environmental background in different population groups.

CONSENT

The authors declare that both informed and written consents were obtained from all participants including cases and controls in their local language.

ETHICAL APPROVAL

The authors declare that the present study was undertaken following the guidelines of Helsinki declaration 1975, revised in 2000 for human studies. The study was undertaken only after getting the written permission from the properly constituted intuitional ethical committee. Both informed and written consent were obtained from all study subjects. All of the data will be strictly maintained and will be kept private as per ethical guidelines and rules.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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