



Original Article

Elevated fasting insulin is associated with cardiovascular and metabolic risk in women with polycystic ovary syndrome

Shayaq Ul Abeer Rasool^a, Sairish Ashraf^b, Mudasar Nabi^b, Fouzia Rashid^c, Khalid Majid Fazili^a, Shajrul Amin^{b,*}^a Department of Biotechnology, University of Kashmir, India^b Department of Biochemistry, University of Kashmir, India^c Clinical Biochemistry, University of Kashmir, India

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ABSTRACT

Aims: PCOS is associated with various immediate and long term health complications. The aim of this study was to investigate the association of serum fasting insulin concentration with cardiovascular and metabolic risk factors in women with polycystic ovary syndrome.

Methods: A total of 349 women, 249 women with polycystic ovary syndrome and 100 age-matched healthy controls, were recruited in this case-control study. Fasting insulin and various other biochemical, hormonal and clinical parameters were measured in all participants. The correlation of insulin with cardiometabolic risk factors was evaluated in PCOS women with normal and high serum insulin concentration.

Results: Fasting Insulin, BMI, WHR, FAI, LH: FSH, HOMA, QUICKI were significantly higher in PCOS women compared with healthy controls ($p < 0.01$). Fasting insulin showed a positive correlation with more cardiovascular and metabolic risk factors in PCOS compared to controls. The BMI, BAI, LAP, HOMA IR, QUICKI and FAI were significantly higher (all $p < 0.05$) in PCOS patients with higher insulin levels than with PCOS women with normal levels.

Conclusion: Fasting insulin is an important determinant in the pathogenesis of obesity and hyperandrogenism in PCOS. It is associated with an increased risk of cardiovascular and metabolic disorders in women with PCOS.

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1. Introduction

Insulin is a central hormone in metabolism with an essential role in glucose and lipid homeostasis. Besides clearing glucose from the bloodstream by facilitating glucose uptake by cells, it stimulates glycogen and fatty acid synthesis in the liver and also inhibits gluconeogenesis, glycogenolysis and lipolysis. The desensitization of target cells towards insulin can promote overexpression and secretion of insulin hence leading to compensatory hyperinsulinemia. Hyperinsulinemia is intricately connected to impaired glucose tolerance (IGT) and insulin resistance (IR). Insulin

resistance is suggested to be a major factor in the pathogenesis of many serious disorders like type 2 diabetes, stroke, cardiovascular diseases, neurodegenerative and malignant disorders (1, 2). Hyperinsulinemia along with impaired glucose tolerance and insulin resistance are frequently found in women with polycystic ovary syndrome (PCOS). PCOS is a widespread multifactorial endocrine disorder, affecting 5–10% of premenopausal women. It is primarily characterized by chronic anovulation, hyperandrogenism and polycystic ovarian morphology [3,4]. Women with PCOS are at increased risk of various reproductive, metabolic, cardiovascular and psychological complications. The primary complaints like menstrual dysfunction, obesity and hirsutism progress into serious long-term complications such as cardiovascular diseases, metabolic syndrome, infertility, type 2 diabetes mellitus, obstructive sleep apnea, obstetrical complications, nonalcoholic steatohepatitis, and psychiatric conditions and reduced quality of life [5–8].

Though etiology is not clearly understood but insulin is

* Corresponding author. Department of Biochemistry, University of Kashmir, Srinagar, 190006, India.

E-mail addresses: shayaq.ul.abeer@gmail.com (S.U.A. Rasool), sairishashraf11@gmail.com (S. Ashraf), mudasarnabi@gmail.com (M. Nabi), rashid.fouzia@gmail.com (F. Rashid), fazili@kashmiruniversity.ac.in (K.M. Fazili), shajrulamin@uok.edu.in (S. Amin).

suggested to play a central role in pathogenesis and persistence of PCOS manifestations. Insulin has an important role in androgen biosynthesis; it stimulates ovarian theca cells and activates cytochrome P450C17 α through MKK3/p38 and MKK4/JNK. Insulin acts as a co-gonadotrophin to increase proliferation in ovarian thecal-interstitial cells by increased phosphorylation of MAPK3/1 to enhance LH- induced androgen synthesis [9]. Insulin resistant and non-insulin resistant PCOS women also show differential expression of PPAR- γ , SREBP1c, FAS, CPT-, pP38MAPK, StAR, CYP19A1, 17 β - HSD and 3 β - HSD [10]. It directly suppresses sex hormone binding globulin (SHBG) synthesis in the liver and participates in PI3K/AKT pathway-mediated systemic insulin resistance and hyperandrogenemia [11,12]. Elevated insulin levels have been found in various metabolic, reproductive and neurodegenerative disorders. But how serum concentration of insulin contributes to pathogenesis and risk of cardiometabolic disorders in PCOS is not completely understood. No study has reported the relationship of serum insulin concentration with various cardiovascular and metabolic risk factors.

The aim of this study was to evaluate the correlation of serum insulin (F) levels with various biochemical and metabolic parameters in PCOS women and age-matched healthy controls. We further investigated the association of different serum insulin concentrations with different cardiovascular and metabolic risk factors in PCOS women.

2. Materials and methods

2.1. Recruitment of subjects

This was a case-control study. A total of 349 women between the age of 16–30 yrs were recruited for the study. The women visiting endocrinology outpatient clinic of Sher e Kashmir Institute of Medical Sciences (SKIMS), Srinagar, India for PCOS-related symptoms such as hirsutism, acne, obesity, infertility or menstrual irregularities from June 2015 to March 2018 were evaluated for PCOS. The diagnosis of PCOS was done according to the revised 2003 European Society for Human Reproduction and Embryology and American Society for Reproductive Medicine (ESHRE/ASRM or Rotterdam) criteria [13] The women were diagnosed with PCOS if at least two of the following three features were present [1]: oligo-ovulation defined by menstrual cycles of >35 days and amenorrhea as absence of at least 6 menstrual cycles [2] clinical and/or biochemical signs of hyperandrogenism, (FerrimanGallaway score ≥ 8 or Total testosterone ≥ 50 ng/dL) and [3] polycystic ovaries; ≥ 12 antral follicles measuring 2–9 mm in diameter or ovarian volume >10 mL in at least one ovary by *trans*-abdominal ultrasonic examination [14]. All subjects were screened to exclude PCOS mimicking disorders like hyperprolactinemia, thyroid dysfunction, Cushing's syndrome, congenital adrenal hyperplasia, and androgen-secreting ovarian/adrenal tumors. The control group consisted of 100 age-matched healthy women volunteers with regular menstrual cycles, no clinical or biochemical hyperandrogenism on physical examination. They also had no history of endocrine or autoimmune disorders and not undergone surgery in the pelvic region. All subjects were ethnic Kashmiris living in Kashmir province and having not received hormonal therapy for at least 3 months before hormonal assays.

2.2. Ethics statement

This study was approved by the institutional ethics committee under Ethical approval no. SIMS 1–31/IEC-SKIMS/2013/6592. Subjects were recruited after written informed consent was obtained from them.

2.3. Anthropometric and clinical evaluation

Detailed clinical history was taken from the participants of the study and their clinical symptoms like menstrual history, acne, alopecia and acanthosis nigricans were recorded. The general anthropometric variables were collected, that included-weight, height, waist-hip ratio, systolic and diastolic blood pressure (SBP and DBP) and extent of hirsutism (FG score ≥ 8 taken as significant). Height was measured in standing position without shoes using a Prestige height measuring scale. Weight was measured by Krups weighing scale with least count of 500 g with light clothing and without shoes. Waist circumference was determined in standing position as the minimum value between the iliac crest and the lateral costal margin at the end of a gentle expiration, and hip circumference was calculated as the maximum value over the buttocks. Blood pressure (BP) was measured in a relaxed sitting position after 5–10 min rest by Diamond Mercurial Sphygmomanometer. The hirsutism was measured by Ferriman-Gallway scoring system.

2.4. Biochemical and hormonal assessment

The blood samples were obtained in clot activator vials from the participants after a 12-h overnight fast in the early follicular phase (Day 2–3) of the spontaneous menstrual cycle or withdrawal bleeding with progesterone for subjects with amenorrhea. The blood vials were placed on ice immediately, serum was separated by centrifugation at 3000 rpm for 10 min at 4 °C within 2 h. Serum aliquots were stored at -80 °C for further analysis.

The fasting insulin concentration was measured by the direct sandwich solid phase two-site enzyme-linked immunosorbent assay kit (Calbiotech US, Cat. No. IS130D). Briefly, serum from PCOS cases and control was incubated for 60 min at 20–25°C with the enzyme (HRP)-conjugated anti-insulin antibody in micro-titration wells coated with anti-insulin antibody. The wells were washed with 1X wash buffer to remove unbound enzyme-labeled antibody and TMB substrate was added to wells to detect bound HRP complex and incubated for 15 min at room temperature. The reaction was stopped by adding acidic stop solution to give a colorimetric endpoint and absorbance was measured at 450 nm. The inter and intra-assay coefficient of variation was 8.1% and 7.4%.

The serum concentration of luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone (TT), thyroid-stimulating hormone (TSH), Prolactin (PRL) was measured by Radioimmunoassay (RIA) on Beckman Coulter UniCelDxI 800 (Access Immunoassay system) using RIA kits (Immunotechs.r.o, Prague, Czech Republic). ELISA was used to measure the serum concentration of androstenedione and dehydroepiandrosterone sulfate (DHEAS) (Calbiotech, CA USA) and sex hormone-binding globulin (SHBG) (DGR Instruments GmbH Marburg). The absorbance was measured in ELISA tests by using SkanIt RE 4.0 software on Thermo Scientific Multiskan FC ELISA reader. The protocol recommended by the manufacturer was followed. The glucose and insulin were measured in fasting state and 2 h post 75 g oral glucose tolerance test (OGTT) in study subjects. The levels of glucose were measured by the glucose oxidase method. The biochemical parameters like cholesterol (CHOL), triglycerides (TG), urea, uric acid, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by enzymatic methods using Erba bioassay diagnostic kits and analyzed on Erba Chem7 biochemistry analyzer (ERBA Diagnostics Mannheim, Germany).

2.5. Measurement of cardiovascular and metabolic risk factors

Body mass index was measured as (BMI: weight [kg]/height

[m²]), Waist-hip ratio was calculated by (WHR: waist circumference [cm]/hip circumference[cm]). The body adipose index (BAI) was calculated using Hip Circumference and height BAI = (HC in centimeters)/(height in meters)^{1.5} - 18).

The free androgen index (FAI) was derived using the formula:

$$FAI = \frac{\text{Total Testosterone(nmol/L)}}{\text{SHBG (nmol/L)}} \times 100$$

The homeostatic model assessment of insulin resistance (HOMA-IR) was derived using the formula: Fasting glucose(mg/dL) x Fasting Insulin (uIU/mL)/405.

The quantitative insulin sensitivity check index (QUICKI) was estimated according to the formula: 1 /logFasting Insulin (uIU/mL) + log Fasting Glucose(mg/dL)

The lipid accumulation product (LAP) was calculated using the formula: [WC-58]xTG, where 58 was the minimum WC value according to the Third National Health and Nutrition Examination Survey (NHANESIII).

2.6. Statistical analysis

All continuous variables were presented as mean ± standard deviation and categorical variables as numbers and percentages. Parametric variables of clinical, hormonal and metabolic variables were compared between PCOS and controls as well as between groups with Insulin<25 and Insulin>25 by unpaired student t-test. Non-parametric variables were compared by chi-square test. One way analysis of variance (ANOVA) independent standard weighted-means analysis was used to compare multiple groups followed by posthoc Bonferroni test for intergroup association. Kruskal Wallis test was used for comparing nonparametric data in multiple groups. The correlation of fasting insulin with cardiovascular and metabolic risk factors was done by Pearson correlation test. The box plots, scatter plots and bar graphs were created using Sigma Plot 10. The statistical analysis was performed using statistical computation software vassarstats (<http://vassarstats.net/>). P-value of <0.05 was considered as statistically significant.

3. Results

The baseline clinical features of PCOS and control women i.e., age (22.43 ± 4.14 vs. 22.01 ± 3.17, p = 0.362), height (1.57 ± 0.05 vs 1.57 ± 0.05, p = 0.518), systolic blood pressure (120.58 ± 7.51 vs 118.96 ± 5.40, p = 0.05), diastolic blood pressure (80.54 ± 5.89 vs 79.32 ± 5.23, p = 0.072) and menarche (13.14 ± 1.14 vs 13.28 ± 1.07, p = 0.292) were comparable. The weight, BMI, Waist Hip ratio (WHR) and FG score were significantly higher in PCOS group as compared to controls (all p < 0.01). The results are summarized in Table 1.

The insulin (F) level was significantly higher (p < 0.001) in PCOS women, median = 12.49uIU/mL; (lower quartile (x_L) = 8.21, upper quartile (x_U) = 18.5; interquartile range (IQR) = 10.29) compared to median insulin level in controls 6.78uIU/mL (lower quartile (x_L) = 3.43, upper quartile (x_U) = 10.66; interquartile range (IQR) = 7.23) (Fig. 1).

The Pearson correlation analysis showed that the insulin (F) in PCOS group had positive correlation with BMI (r = 0.358, p < 0.001), WHR (r = 0.133, p < 0.05), WHtR (r = 0.376, p < 0.001) glucose F (r = 0.181, p < 0.001), FAI (r = 0.141, p < 0.05), FG Score (r = 0.176, p < 0.005) Testosterone (r = 0.227, p < 0.001), HOMA IR (r = 0.092, p < 0.001) and LAP (r = 0.337, p < 0.001) while it showed negative correlation with QUICKI (r = -0.900, p < 0.001). In contrast, the insulin (F) levels in control group had positive correlation with WHR (r = 0.207, p < 0.05), Testosterone (r = 0.251, p = 0.012), HOMA IR

Table 1

Clinical, biochemical and hormonal status of PCOS cases and Control groups.

Parameter	PCOS (n = 249)	Controls (n = 100)	P value
Age (years)	22.43 ± 4.14	22.01 ± 3.17	0.362
Weight (kg)	59.83 ± 11.59	51.94 ± 6.72	<0.001*
Height (m)	1.57 ± 0.05	1.57 ± 0.05	0.518
BMI (kg/m ²)	24.28 ± 4.69	21.16 ± 2.48	<0.001*
Waist (cm)	83.10 ± 11.13	77.01 ± 7.11	<0.001*
Hip (cm)	93.47 ± 8.24	91.30 ± 6.33	0.0185*
WHR	0.89 ± 0.08	0.84 ± 0.06	<0.001*
WHtR	0.53 ± 0.07	0.49 ± 0.04	<0.001*
BAI	29.58 ± 4.73	28.62 ± 3.04	0.061
SBP (mmHg)	120.58 ± 7.51	118.96 ± 5.40	0.0505
DBP (mmHg)	80.54 ± 5.89	79.32 ± 5.23	0.072
Menarche (years)	13.14 ± 1.14	13.28 ± 1.07	0.292
FG Score	13.95 ± 6.58	4.53 ± 1.84	<0.001*
LH (IU/L)	11.29 ± 9.54	6.65 ± 2.35	<0.001*
FSH (IU/L)	6.09 ± 1.87	6.90 ± 1.97	<0.001*
TT (ng/dL)	61.09 ± 23.01	34.15 ± 15.60	<0.001*
PRL (ng/mL)	13.29 ± 5.61	10.42 ± 4.93	<0.001*
TSH (uIU/L)	3.18 ± 1.44	2.99 ± 1.47	0.268
SHBG (nmol/L)	50.07 ± 21.67	64.91 ± 25.42	<0.001*
Andro (ng/mL)	3.26 ± 0.86	2.26 ± 0.69	<0.001*
DHEAS (ng/mL)	3.79 ± 1.15	2.9 ± 1.3	<0.001*
Insulin F (uIU/ml)	13.53 ± 7.19	7.72 ± 5.39	<0.001*
Glu F (mg/dL)	85.70 ± 8.59	84.54 ± 8.87	0.202
Glu 2 h (mg/dL)	115.47 ± 18.01	108.55 ± 14.29	<0.001*
Chol (mg/dL)	154.73 ± 34.64	135.11 ± 19.30	<0.001*
TG (mg/dL)	120.59 ± 35.57	102.73 ± 14.78	<0.001*
HOMA IR	2.83 ± 1.68	1.62 ± 1.36	<0.001*
QUICKI	0.338 ± 0.02	0.38 ± 0.05	<0.001*
FAI	5.77 ± 5.62	2.21 ± 1.66	<0.001*
LH:FSH	1.91 ± 1.27	1.04 ± 0.54	<0.001*
LAP	35.16 ± 21.81	22.20 ± 9.08	<0.001*
Urea (mg/dL)	22.76 ± 5.97	21.32 ± 3.47	0.024
UA (mg/dL)	4.27 ± 1.09	3.84 ± 0.75	<0.001*
Creatinine (mg/dL)	1.03 ± 0.43	0.80 ± 0.13	<0.001*
AST (U/L)	31.39 ± 12.46	18.30 ± 7.84	<0.001*
ALT (U/L)	27.84 ± 13.82	23.46 ± 6.78	0.002*

Data presented as Mean ± SD. *P-value <0.05 significant. P values calculated by Independent Student's t-test.

PCOS polycystic ovary syndrome, BMI body mass index, WHtR Waist to height ratio, BAI body adiposity index, SBP systolic blood pressure, DBP diastolic blood pressure, FG Score ferrimangallwey score, LH luteinizing hormone, FSH follicle stimulating hormone, TT total testosterone, PRL Prolactin, TSH thyroid stimulating hormone, SHBG sex hormone binding globin, Androandrostendione, DHEASdihydroepian-drostenedionesulphate, Glu F glucose fasting, CHOL cholesterol, TG triglycerides, HOMA IR homeostasis model assessment-estimated insulin resistance, QUICKI quantitative insulin sensitivity check index, FAI free androgen index, LAP lipid accumulation product, UA uric acid, AST aspartate aminotransferase, ALT alanine aminotransferase.

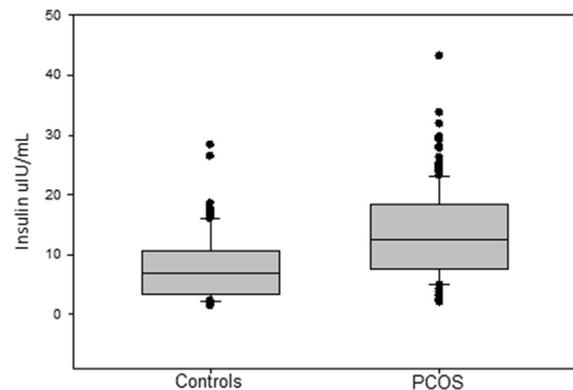


Fig. 1. Box plot illustrating the median and interquartile range of Insulin levels in controls and PCOS women. Boundaries of the box signify the lower and upper quartiles.

($r = 0.846$, $p < 0.001$) and LAP ($r = 0.230$, $p < 0.05$) but showed a negative correlation between FG score ($r = -0.233$, $p < 0.05$) and QUICKI ($r = -0.666$, $p < 0.001$). Also, no correlation was found between fasting insulin and LH:FSH in PCOS cases. There was no correlation of fasting insulin with BMI, LH:FSH, glucose (F), cholesterol and FAI in controls. The results are given in Table 2.

Using the Langer et al. [15], definition for insulin resistance, the concentration of fasting insulin was cutoff value and PCOS patients were categorized in three groups (a) Normal (Insulin $< 13 \mu\text{IU/mL}$), (b) Intermediate (Insulin $13\text{--}25 \mu\text{IU/mL}$), (c) High (Insulin $> 25 \mu\text{IU/mL}$). The comparison of clinical, biochemical and cardiovascular-metabolic risk factors showed high group had a significantly higher proportion of women with acne (69.23%), alopecia (53.84%) and amenorrhea (23.07) as compared with normal and intermediate groups. The high group had significantly higher acanthosis nigricans (69.23%) and obese women (61.53%). PCOS women in the intermediate group were mostly overweight (40.54%) and in normal group, PCOS women included women with normal BMI (Table 3).

The weight, waist, hip, testosterone and fasting glucose were significantly higher ($P < 0.01$) in Intermediate and High group than Normal group. There was no statistically significant difference in height, FG Score, SBP, DBP, LH, FSH, PRL, TSH, SHBG, Androstenedione, DHEAS, CHOL, TG, Glu (2 h), liver and kidney marker enzyme between the groups. The cardiovascular-metabolic risk factors BMI ($p < 0.001$), WHtR ($p < 0.001$), HOMA IR ($p < 0.001$), FAI ($p < 0.05$) and LAP ($p < 0.05$) were significantly higher in groups with higher insulin levels. WHR ($p = 0.274$) and LH:FSH ($p = 0.419$) were not significantly different among the groups. The FG score was also comparable ($p = 0.183$) between the groups (Table 4).

The Pearson correlation analyses showed that HOMA IR, QUICKI, LAP had a significant positive correlation (all $p < 0.05$) with fasting insulin levels in all three groups. The FGS was positively correlated in Insulin < 13 group ($r = 0.018$, $p = 0.044$) and Insulin > 25 group ($r = 0.547$, $p = 0.050$). BMI, WHR, WHtR and Glu (F) were positively correlated with insulin in normal and intermediate groups but no significant correlation was found with insulin for these markers in High group. FAI was correlated with insulin in the normal group ($r = 0.181$, $p = 0.043$) but not in intermediate and High group. Cholesterol had significant positive correlation with insulin in intermediate ($r = 0.905$, $p < 0.001$) and High ($r = 0.605$, $p = 0.02$) groups but not in normal group (Table 5).

4. Discussion

The novel findings of the present study are that serum levels of fasting insulin were significantly correlated with various markers of cardiovascular and metabolic dysfunction. In PCOS women with higher serum insulin concentrations, these parameters were significantly increased. This shows that fasting insulin is associated with increased cardiometabolic risk in PCOS women.

PCOS, being a multisystemic disorder, is not just associated with

chronic anovulation and clinical manifestations of hyperandrogenism (hirsutism, acne and androgenic alopecia) but is also associated with wide range of long-term cardiovascular, metabolic and psychological complications [16,17]. Hyperinsulinemia, an immediate consequence of insulin resistance (IR), is proposed as a fundamental factor in the pathogenesis of long term complications. IR has been consistently reported symptom in PCOS independent of BMI [18,19]. Our data has confirmed that hyperinsulinemia is implicated in the pathogenesis and associated with various risk factors that lead to long term PCOS related complications. We show hyperinsulinemia tends to increase fats accumulation and lead to central adiposity. Insulin also enhances androgen production, its bioavailability in target tissues and is involved in derangement of various physiological processes in PCOS women which combine to drive a network of events which finally lead to the development of PCOS.

Our results show women with PCOS have significantly higher levels of various CVD and metabolic risk predictors like BMI, WHR, BAI, LH: FSH, HOMA IR, LAP, ALT, AST, etc. ($p < 0.01$) and lower insulin sensitivity, QUICKI ($p < 0.001$) compared to healthy women. The influence of hyperinsulinemia and insulin resistance on CVD and metabolic risk has been reported in several studies. In our study, PCOS women with higher fasting insulin ($> 13 \mu\text{IU/mL}$) exhibited higher values of TG, Chol, LAP, HOMA, QUICKI, and BAI than in PCOS women with normal insulin F levels.

In PCOS women, insulin was positively correlated with cardiovascular and metabolic risk factors than healthy controls. This shows insulin play more complex role PCOS than in healthy women. This can be because hyperinsulinemia and insulin resistance are involved in derangement of hormonal and metabolic balance in the body, and set into action a vicious cycle of processes that drive each other.

Various studies were carried out to determine fasting insulin cutoff values to screen and understand the relation with insulin resistance. Lee et al. [20], found that the cutoff value of fasting insulin for determining the insulin resistance was $12.94 \mu\text{IU/mL}$ by estimating the areas under the receiver-operating characteristic (ROC) curve. Lunger et al. [15] in 263 PCOS women found that $> 13 \mu\text{IU/mL}$ gave the diagnosis in 70% for insulin resistance and Carmina et al. [21] defined $< 12 \mu\text{IU/mL}$ as normal insulin sensitivity in a study with 276 PCOS women. Our results also showed that the $> 13 \mu\text{IU/mL}$ cutoff was significantly associated with cardiometabolic risk factors than women with $< 13 \mu\text{IU/mL}$ fasting insulin. A study with Asian Indian females ($n = 174$) showed 75th percentile cutoff for insulin was $25 \mu\text{IU/mL}$ [22]. Our study found women in High insulin group were at significantly higher risk of developing cardiovascular and metabolic disorders.

Previous studies have shown anthropometric parameters are strongly associated with cardiovascular and metabolic risk. BMI has been long considered as a decisive risk factor for development and progression of cardiovascular and metabolic disorders [23,24]. Our data showed PCOS women had significantly higher weight and BMI

Table 2

Correlation of fasting insulin with cardiovascular and metabolic risk factors in PCOS and control groups.

		BMI	WHR	WHtR	FGS	TESTO	GLU (F)	CHOL	HOMA IR	QUICKI	FAI	LAP
PCOS	r	.358**	.133*	.376**	.176**	.227**	.181**	.179**	.092**	-.900**	.141**	.337**
	P	0.000	.036	.001	.005	.000	.004	.005	.000	.000	.027	.000
CONTROLS	r	.122	.207*	.238	-.233*	.251*	.019	-.008	.846**	-.666**	.116	.230
	P	.229	.040	.016*	.020	.012	.854	.941	.000	.000	.252	.02*

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Data are presented as the Pearson correlation coefficient r and P value. BMI body mass index, WHR waist-hip ratio, WHtR Waist to height ratio, FGS ferriman gallwey score, TESTO total testosterone, GLU (F) fasting glucose, GLU (2 h) 2nd-hour glucose, CHOL cholesterol, HOMA IR homeostasis model assessment-estimated insulin resistance, FAI free androgen index, LAP lipid accumulation product.

Table 3
Clinical features and cutaneous manifestations in different groups.

Parameter	Normal (n = 125)	Intermediate (n = 111)	High (n = 13)	(H) P value
Cycles				
Regular	(20) 16.00%	(15) 13.51%	(1) 7.69%	
Amenorrhoea	(11) 8.80%	(23) 20.72%	(3) 23.07%	
Oligomenorrhoea	(94) 75.20%	(73) 65.76%	(9) 69.23%	(3.334) 0.189
Acne	(69) 55.20%	(64) 57.65%	(9) 69.23%	(0.716) 0.699
Alopecia	(43) 34.40%	(47) 42.34%	(7) 53.84%	(2.016) 0.364
Acanthosis	(25) 20.00%	(49) 44.14%	(9) 69.23%	(15.52) 0.000*
Hirsutism	(104) 83.2%	(86) 77.47%	(10) 76.92%	(0.09) 0.956
BMI				
<18.5	(17) 13.60%	(14) 12.61%	(0) 00%	
18.6–25	(72) 57.60%	(35) 31.53%	(1) 7.69%	
25.01–29.99	(30) 24.00%	(45) 40.54%	(4) 30.76%	
>30.00	(6) 4.8%	(17) 15.31%	(8) 61.53%	(28.63) <0.000*

Data is presented as (number)percentage.

BMI body mass index, (H) P H statistic P value of Kruskal-Wallis test.

Table 4
Comparison of different biochemical and cardiometabolic parameters in PCOS patients with different insulin concentrations.

Parameter	Normal (n = 125)	Intermediate (n = 111)	High (n = 13)	P value
Age (years)	21.90 ± 4.17	22.80 ± 4.08	24.62 ± 3.64	0.036*
Weight (kg)	56.76 ± 9.41a ^{ab*}	61.74 ± 12.64 ^{c*}	73.08 ± 8.66	<0.001*
Height (m)	1.57 ± 0.05	1.57 ± 0.06	1.55 ± 0.04	0.274
BMI (kg/m ²)	22.96 ± 3.88 ^{ab*}	25.06 ± 4.93 ^{c*}	30.45 ± 3.81	<0.001*
Waist (cm)	80.41 ± 9.15 ^{ab*}	84.99 ± 12.23 ^c	92.85 ± 10.64	<0.001*
Hip (cm)	91.54 ± 7.40 ^{b*}	94.70 ± 8.53 ^c	101.54 ± 7.26	<0.001*
WHR	0.88 ± 0.08	0.90 ± 0.08	0.91 ± 0.05	0.175
WHtR	0.51 ± 0.06 ^{ab*}	0.54 ± 0.08 ^c	0.60 ± 0.08	<0.001*
BAI	28.43 ± 4.22 ^{ab*}	30.27 ± 4.74 ^{c*}	34.72 ± 4.91	<0.001*
SBP (mmHg)	120.05 ± 6.06	6120.72 ± 8.81 ^{c*}	124.23 ± 5.71	0.148
DBP (mmHg)	80.44 ± 5.03 ^{b*}	80.62 ± 6.55	84.23 ± 5.72	0.080
Menarche (years)	12.97 ± 1.16	13.30 ± 1.11	13.46 ± 0.88	0.048*
FG Score	13.28 ± 6.21	14.45 ± 6.88	16.15 ± 7.16	0.183
LH (IU/L)	10.97 ± 6.80	11.78 ± 12.23	10.25 ± 5.34	0.748
FSH (IU/L)	6.34 ± 1.89	5.81 ± 1.83	5.99 ± 1.73	0.088
TT (ng/dL)	55.49 ± 21.13 ^{ab*}	64.49 ± 23.33 ^{c*}	85.87 ± 15.61	<0.001*
PRL (ng/mL)	13.54 ± 5.53	12.89 ± 5.81	14.33 ± 4.61	0.535
TSH (uIU/L)	3.29 ± 1.68	3.09 ± 1.18	2.93 ± 0.86	0.474
SHBG (nmol/L)	51.78 ± 22.96	49.52 ± 20.42	38.47 ± 15.71	0.100
Andro (ng/mL)	3.26 ± 0.87	3.19 ± 0.86 ^{c*}	3.73 ± 0.57	0.096
DHEAS (ng/mL)	3.79 ± 1.28	3.81 ± 1.01	3.53 ± 0.98	0.707
Glu F (mg/dL)	84.84 ± 7.45 ^{b*}	85.80 ± 9.32 ^c	93.08 ± 9.40	0.004*
Glu 2 h (mg/dL)	114.53 ± 19.18	115.89 ± 17.06	120.85 ± 13.75	0.459
Chol (mg/dL)	150.01 ± 29.61	159.55 ± 39.36	158.92 ± 32.76	0.096
TG (mg/dL)	118.58 ± 33.71	123.90 ± 38.00	111.77 ± 30.67	0.340
HOMA IR	1.68 ± 0.63 ^{ab*}	3.81 ± 0.96 ^{c*}	6.91 ± 1.50	<0.001*
QUICKI	0.345 ± 0.01 ^{ab*}	0.316 ± 0.01 ^{c*}	0.291 ± 0.01	<0.001*
FAI	4.89 ± 3.63 ^b	6.36 ± 7.14	9.17 ± 4.90	0.010*
LH:FSH	1.81 ± 1.08	2.02 ± 1.47	1.84 ± 1.04	0.419
LAP	30.55 ± 16.86 ^{ab}	39.16 ± 25.46	45.10 ± 21.82	0.002*
Urea (mg/dL)	22.27 ± 6.03	22.85 ± 5.96	26.38 ± 4.29	0.058
UA (mg/dL)	4.37 ± 1.13	4.15 ± 1.05	4.29 ± 0.89	0.294
Creatinine (mg/dL)	1.02 ± 0.47	1.05 ± 0.40	0.90 ± 0.28	0.459
AST (U/L)	31.63 ± 13.54	30.50 ± 10.76	36.77 ± 14.63	0.219
ALT (U/L)	28.57 ± 15.99	26.90 ± 10.99	28.85 ± 13.53	0.628

Data presented as Mean ± SD. *P-value <0.05 significant.

P values calculated by one-way Analysis of Variance (Anova) Independent standard weighted-means analysis.

Inter-group association tested by Post hoc Tukey HSD test and shown as.

a Insulin <13 vs Insulin 13–25 P < 0.05, a* P < 0.01.

b Insulin <13 vs Insulin >25 P < 0.05, b* P < 0.01.

c Insulin 13 - 25 vs Insulin >25 P < 0.05, c* P < 0.01.

PCOS polycystic ovary syndrome, BMI body mass index, WHtR Waist to height ratio, SBP systolic blood pressure, DBP diastolic blood pressure, FG Score ferriman gallwey score, LH luteinizing hormone, FSH follicle stimulating hormone, TT total testosterone, PRL Prolactin, TSH thyroid stimulating hormone, SHBG sex hormone binding globin, Androandrostenedione, DHEASdihydroepiandrosteronesulphate, Glu F glucose fasting, CHOL cholesterol, TG triglycerides, HOMA IR homeostasis model assessment-estimated insulin resistance, QUICKI quantitative insulin sensitivity check index, FAI free androgen index, LAP lipid accumulation product UA uric acid, AST aspartate aminotransferase, ALT alanine aminotransferase.

than healthy controls. The BMI was positively correlated with insulin and PCOS women with high insulin had significantly higher BMI than PCOS women with lower insulin. The anatomical

distribution of adipose tissue is suggested to be of far greater importance as a predictor of cardiometabolic risk. In fact, markers of central adiposity like WHR, WHtR and BAI are a stronger

Table 5

Pearson correlation of fasting insulin with cardiovascular and metabolic risk factors in groups with different insulin concentrations.

Parameters	Normal		Intermediate		High	
	r	P	r	P	r	P
BMI	0.22*	0.013	0.208*	0.028	0.341	0.255
WHR	0.309**	0.000	0.114*	0.032	0.217	0.474
WHtR	0.285**	0.001	0.196*	0.038	0.367	0.216
FGS	0.018*	0.044	0.156	0.101	0.547*	0.050
GLU (F)	0.276**	0.001	0.312**	0.000	0.263	0.382
CHOL	0.10	0.264	0.292**	0.001	0.605*	0.020
HOMA IR	0.962**	0.000	0.905**	0.000	0.888**	0.000
QUICKI	0.497**	0.000	−0.895**	0.000	−0.791**	0.000
FAI	0.181*	0.043	−0.111	0.244	−0.05	0.845
LAP	0.207*	0.020	0.361**	0.000	0.657**	0.001

r Pearson correlation coefficient, P two tailed p value of correlation.

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Data is presented as Pearson correlation coefficient r and p-value.

BMI body mass index, WHR waist-hip ratio, WHtR Waist to height ratio, FGS Ferriman gallwey score, GLU (F) fasting glucose, CHOL cholesterol, HOMA IR homeostasis model assessment-estimated insulin resistance, QUICKI quantitative insulin sensitivity check index, FAI free androgen index, LAP lipid accumulation product.

determinant of cardiometabolic risk. In the present study, WHR was higher ($p < 0.001$) in PCOS women than controls and were positively correlated with insulin (F) levels. When compared between normal and increased insulin concentrations groups, we found waist, hip circumferences were significantly elevated among insulin 13–25, insulin >25 than insulin <13 groups. WHtR is suggested to be an early indicator and best predictor of cardiovascular risk [25,26]. We found that PCOS women had significantly higher ($p < 0.001$) WHtR than healthy women. The WHtR was positively related to Insulin (F) concentration and was found to increase significantly with insulin concentration. The WHtR is a proxy marker of visceral adipose tissue and a strong cardiometabolic risk factor [27,28]. Nambiar et al. [29] showed WHtR is a more reliable marker than BMI for pediatric metabolic syndrome and associated with several components of MetS. A cross-sectional study in Singapore reported BAI was the best predictor of CVD risk in females [30]. We found BAI was significantly higher in PCOS women with high insulin levels. Some studies have indicated that LAP index can be a better predictor of cardiovascular and metabolic risk than BMI, BAI or WHR [31,32]. Our data shows PCOS women had significantly higher LAP index than healthy controls ($p < 0.01$). LAP value was significantly higher in insulin >25 group showing that insulin plays a major role in central adiposity. This can be explained by the role of insulin in dysregulation of lipid metabolism by affecting triglyceride levels and influencing lipid metabolism. The data suggest that high WHR, WHtR, BAI and LAP contribute to the pathophysiology by enhancing abdominal adiposity and also contributing to insulin resistance. The insulin contributes to lipid accumulation stimulating differentiation of preadipocytes to adipocytes via Akt-TSC2-mTORC1 Pathway [33], by inhibiting of lipolysis through increasing steroid regulatory element-binding protein (ADD-1/SREBP-1a) transcription factor [34,35] and inhibition of enzyme hormone-sensitive-lipase (HSL) [36]. Increased lipid storage is also due to an increase in liver-specific fatty acid transporter proteins (FATP) and mediated via IRS signaling [37]. Studies have shown hyperinsulinemia drives diet-induced obesity [38] and its attenuation provides long-lasting protection against obesity [39]. We found CHOL and TG are significantly elevated in PCOS women than controls. Further, we found that cholesterol significantly correlated with intermediate and high insulin groups. This suggests hypertriglyceremia and hypercholesterolemia are involved in PCOS pathway and are significantly influenced by insulin

concentrations. These observations show that insulin has a significant influence on the accumulation of lipids, abdominal adiposity and hence predisposes PCOS women to obesity, particularly central adiposity. This is confirmed by our results where we found PCOS women with high insulin levels comprised overweight and obese women as compared to those women having lower insulin concentration had normal BMI.

Previous studies indicate hyperandrogenism, the cardinal feature of PCOS, plays an important role in increasing cardiometabolic risk [40]. Yang et al., showed HA increased the risk of metabolic disorders in women with PCOS [41]. We found fasting insulin showed a strong positive correlation with testosterone. The PCOS women with increased insulin concentration showed significantly higher testosterone levels, confirming that insulin indeed drives androgen production. We also found significantly lower SHBG level in PCOS women than controls but there was no significant reduction in SHBG level in PCOS women with higher insulin concentration than normal insulin levels. This shows that insulin shows a more pronounced effect on testosterone production than affecting the level of SHBG. Low levels of SHBG contribute high free testosterone by reduced testosterone binding. This was reflected in FAI value which was significantly higher in women with PCOS. FAI values were positively correlated with insulin concentrations and were significantly higher in the insulin >25 group than insulin <13 group. Our results are in accordance with studies that show insulin lowering or sensitizing agents improve hyperandrogenemia with a decrease in insulin concentration [42–44].

The insulin increases androgen biosynthesis in human adrenal cells and is suggested to increase baseline androgenesis. Kinyua et al. [45] reported insulin influences adrenal steroidogenesis by increasing expression of steroidogenic factor 1 (SF-1) through inhibition of FoxO1. We found ANDRO and DHEAS significantly increased in PCOS than controls but in PCOS women with high insulin levels, DHEAS was lower than insulin <13 group and overall difference between androstenedione and DHEAS were not statistically significant. The studies have revealed a complex relation between adrenal androgens.

The insulin resistance is extensively reported as strong markers of CVD and metabolic anomalies. The decreased cellular response to insulin or desensitization to insulin signals basically leads to compensatory hyperinsulinemia. Our PCOS subjects showed significantly higher HOMA than healthy controls and PCOS group with high insulin exhibited higher HOMA values. Insulin resistance finally leads to impaired glucose tolerance and dysglycemia. Though our results show PCOS have comparable fasting glucose level with healthy controls but the PCOS women with high insulin level had significantly higher fasting glucose than groups with normal and intermediate insulin level. This persistent dysglycemia can further lead to hyperglycemia and T2DM. One of the fallout of hyperglycemia and hyperandrogenism is oxidative stress, which is implicated in various cardiometabolic and reproductive complications.

We found PCOS women with high insulin concentration did not show a significant increase in glucose (2 h), liver and kidney marker enzymes. This can be due to the younger age of our study subjects and because many of the effects of hyperinsulinemia are slow and complications like T2DM, MetS, and CVD may take years to accumulate. Liang et al., reported that clinical signs of hyperandrogenism resolve with age but metabolic and other long term effects become more prevalent with increasing age [46]. The PCOS group with high insulin concentrations was of higher age than the other two groups. This also shows hyperinsulinemia/insulin resistance progresses with age. This brings into focus the need to evaluate the fasting insulin levels in PCOS women so that timely management with drugs like insulin sensitizers, diet and exercise

can help to ameliorate the effect of high insulin levels and also decrease the risk of secondary complications associated with PCOS.

5. Conclusion

Our study shows that women with PCOS have increased basal serum insulin secretion and at tissue level show selective insulin resistance for metabolic but not mitogenic pathways. Hyperinsulinemia plays a central role in unifying multiple factors involved in the pathogenesis of PCOS and the severity of its symptoms. Women with high fasting insulin levels show significantly higher values of markers of cardiovascular and metabolic risk which puts such women at a strong susceptibility for developing long term cardiometabolic complications. Therefore our findings show that insulin concentration can be an early marker for insulin resistance, metabolic dysfunction and increase in the risk of development of CVD. This brings into focus the role of managing hyperinsulinemia and using strategies in clinical practice that lead to enhanced insulin sensitivity which in turn can help in achieving better management of PCOS related long term complications.

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Conflicts of interest

Authors declare no conflict of interest.

Consent for publication

All authors have approved the manuscript for submission.

Authors' contributions

Rasool SA: Major work done, Manuscript Writing, Project Development.

Ashraf S: Recruitment of Participants, Data Collection, Critical Revision of Manuscript.

Nabi M: Sample collection, Data Collection, Manuscript Editing and Revision.

Rashid F: Project Management, Manuscript revision.

Amin S: Conception and design, Manuscript writing, Project development, Management and Funding.

All authors read and approved the final draft.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dsx.2019.05.003>.

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