

Cardiovascular Risk Factors Insulin Resistance [IR], High Sensitivity C-reactive Protein (hs-CRP) and Fibrinogen in Pre-Menopausal women with Poly cystic ovarian syndrome (PCOS)

Praveen Sharma¹, Aditi Gupta², Purvi Purohit^{3*}

¹Professor & Head, Department of Biochemistry, AIIMS Jodhpur, India.

²Department Biochemistry, SMS Jaipur, India.

³Assistant Professor, Department of Biochemistry, AIIMS Jodhpur, India.

ABSTRACT

Polycystic ovarian syndrome (PCOS) is one of the commonest endocrine pathies in women of reproductive age. We aimed to establish cardiovascular (CVD) risk factors in pre-menopausal Indian women with PCOS. **Methods and Results:** Study included 150 women (100 PCOS and 50 controls) from reproductive age (25-45 years). Both study groups were examined and analysed for anthropometric, clinical and biochemical parameters. Observations were subjected to statistical analysis using the descriptive statistics, Pearson's coefficient of correlation and Multiple regression analysis. PCOS women presented with central adiposity (Waist circumference (cms) - 85.82 ± 12.16 v/s 78.84 ± 9.66 p<0.001), hypertension (mm of Hg) (Systolic blood pressure- 130.89 ± 7.63 v/s 123.3 ± 8.02 ; Diastolic blood pressure- 87.56 ± 5.77 v/s 83.14 ± 6.38 p<0.001), insulin resistance (IR) (6.99 ± 3.10 v/s 4.73 ± 3.66 p<0.001), hyperinsulinemia (μ IU/ml) (29.02 ± 8.85 v/s 19.46 ± 10.80 p<0.001), dyslipidemia and raised luteinizing hormone (LH)(IU/L) (7.52 ± 2.39 v/s 6.18 ± 1.29 p<0.001), fibrinogen (mg/dl) (215.7 ± 58.87 v/s 182.3 ± 14.61 p<0.001) and high sensitivity C-reactive protein (hs-CRP) (mg/L) (2.27 ± 0.95 v/s 2.17 ± 0.30 p<0.001) as compared to healthy control women. 66% PCOS women had metabolic syndrome (MS). Strong association of central adiposity with serum insulin ($r=0.22$; $p=0.007$) and IR with body mass index [BMI], Waist to Hip ratio, hypertension, fibrinogen and hs-CRP was observed. IR ($p<0.005$) was strongest CVD risk factor in these women ($p<0.001$). **Conclusion:** Obesity, IR, high pro-thrombotic and pro-inflammatory factors in Indian PCOS women indicate strong CVD risk and could cause cardiovascular complications in later life.

Key words: Dyslipidemia, fibrinogen, HDL2c, hs-CRP, Insulin resistance, PCOS, sd LDLc.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a heterogeneous disorder affecting approximately 5 to 10% of women of reproductive age worldwide and 52% women of Indian sub-continent origin in UK.¹⁻³ It is a pluri-metabolic syndrome associated with type 2 diabetes mellitus (DM), hypertension (HT), impaired glucose tolerance (IGT) and dyslipidemia^{4,5} collectively termed as metabolic cardiovascular syndrome

(MCS)⁶ and prevention of future cardiovascular adverse effects is needed. Considering, evidence for cardiovascular events in women who were affected by PCOS during fertile age is limited, we planned to evaluate the CVD risk factors that might be associated with PCOS women of our region.

METHODS

The present study included 150 women (100 PCOS and 50 controls) of Indian origin (age group 25-45 years) reporting to the Obstetrics and Gynaecology Clinic of the SMS hospital, Jaipur. Of the total subjects with PCOS, 73% were aged <35 years and 27% were aged >35 years; 85% were married and 15% were unmarried.

***Corresponding address:**

Dr. Purvi Purohit

Dept of Biochemistry AIIMS Jodhpur Basni Phase II,
Jodhpur, India.

E mail: dr.purvipurohit@gmail.com

DOI: 10.5530/jcdr.2015.2.4

Table 1: Anthropometric and Biochemical characteristics of current study

	Control (n=50)	PCOS (n=100)	CI (95%)	p value
	Mean \pm SD	Mean \pm SD		
WC (cms)	78.84 \pm 9.66	85.82 \pm 12.16	3.06 to 10.90	<0.001*
WHR	0.86 \pm 0.07	0.91 \pm 0.07	6.77 to 1.62	0.002§
BMI (kg/m ²)	26.10 \pm 4.48	26.65 \pm 4.69	2.13 to -1.03	0.493
SBP (mm of Hg)	123.3 \pm 8.02	130.89 \pm 7.63	4.92 to 10.25	<0.001*
DBP (mm of Hg)	83.14 \pm 6.38	87.56 \pm 5.77	2.36 to 6.47	<0.001*
FBS (mg/dl)	90.90 \pm 19.36	96.36 \pm 23.47	-2.16 to 13.08	0.15
PP blood sugar (mg/dl)	110.19 \pm 19.64	124.31 \pm 32.26	2.39 to 4.30	0.005§
HbA _{1c} (%)	2.73 \pm 1.14	3.04 \pm 1.39	-0.14 to 0.75	0.17
Insulin (μ IU/ml)	19.46 \pm 10.80	29.02 \pm 8.85	6.28 to 12.81	<0.001*
HOMA-IR	4.73 \pm 3.66	6.99 \pm 3.10	1.12 to 3.38	<0.001*
TC (mg/dl)	149.57 \pm 27.26	151.84 \pm 10.8	-3.80 to 8.34	0.46
TG (mg/dl)	115.06 \pm 37.97	139 \pm 62.93	4.18 to 42.61	0.017§
HDLc (mg/dl)	45.15 \pm 6.49	40.63 \pm 7.08	-6.88 to -2.15	<0.001*
HDL-2C (mg/dl)	23.04 \pm 7.60	20.15 \pm 3.89	0.87 to 8.55	0.017§
HDL-3C (mg/dl)	30.03 \pm 3.81	30.55 \pm 3.30	-0.67 to 1.71	0.38
LDLc (mg/dl)	105.84 \pm 22.27	106.40 \pm 39.27	-11.28 to 12.39	0.92
sd-LDLc (mg/dl)	28.69 \pm 12.35	37.77 \pm 18.94	3.24 to 14.91	0.002§
TG/HDL	2.67 \pm 1.17	3.58 \pm 1.92	0.32 to 1.49	0.002§
TC/HDL	3.39 \pm 0.91	3.86 \pm 0.76	0.19 to 0.74	0.001§
FSH (IU/L)	4.45 \pm 1.46	4.52 \pm 1.40	-0.41 to 0.55	0.77
LH (IU/L)	6.18 \pm 1.29	7.52 \pm 2.39	0.62 to 2.05	<0.001*
Fibrinogen(mg/dl)	182.3 \pm 14.61	215.7 \pm 58.87	16.67 to 50.12	<0.001*
hs-CRP (mg/L)	2.17 \pm 0.30	2.27 \pm 0.95	-0.17 to 0.37	0.74

§ significant ; § very significant ; * Highly significant

Selection criteria

Healthy controls were young females of age group 25 to 45 years with regular menses and no clinical or lab findings of hyperandrogenism.

Diagnostic criteria for PCOS

- Oligo/amenorrhea.
- Clinical or lab findings of hyperandrogenism.
- Ultra-sonographic findings as per Rotterdam consensus workshop group 2004.

Exclusion criteria

- Age < 25 years or > 45 years.
- Pregnancy.
- Hypothyroidism.
- Cushing syndrome.
- Hyper-prolactinemia.
- Non-classical congenital adrenal hyperplasia.
- Use of oral contraceptives, glucocorticoids, anti-androgens, ovulation induction agents, anti-diabetic and

anti-obesity drugs or other hormonal drugs.

Venous blood samples were collected after an overnight fast during the early follicular phase.

Biochemical Analyses

- Fasting blood glucose (FBG) (GOD-PAP)
- Glycosylated Hb (HbA_{1c})
- Triglycerides (TG) (GPO-PAP)
- Total cholesterol (TC) (CHO-PAP)
- High density lipoprotein (HDLc) (Direct homogenous)
- Low density lipoprotein (LDLc) (Direct homogenous)
- Sd LDLc (Precipitation method)
- Insulin (ELISA)
- HOMA – IR
- Follicular stimulating hormone (FSH) (Chemiluminiscence)
- Luteinizing hormone (LH) (Chemiluminiscence)
- Fibrinogen

Table 2: Correlative analyses of PCOS women

	WC	WHR	BMI	Insulin	HOMA-IR
BMI	0.66 0.0001*	0.43 0.0001*	1	0.12 0.12	0.2 0.012 ^s
WC	1	0.64 0.0001*	0.66 0.0001*	0.20 0.01 ^s	0.24 0.002 ^s
WHR	0.64 0.0001*	1	0.43 0.0001*	0.22 0.007 ^s	0.179 0.029 ^s
SBP	0.24 0.002 ^s	0.15 0.052	0.1 0.18	0.15 0.052	0.141 0.84
DBP	0.22 0.006 ^s	0.09 0.24	0.11 0.17	0.105 0.201	0.15 0.06
FBS	0.23 0.005 ^s	0.067 0.41	0.23 0.004 ^s	0.36 0.0001*	0.75 0.0001*
Insulin	0.20 0.01 ^s	0.22 0.007 ^s	0.12 0.12	1	0.86 0.0001*
HOMA-IR	0.24 0.002 ^s	0.17 0.02 ^s	0.20 0.012 ^s	0.86 0.0001*	1
TG	0.39 0.0001*	0.27 0.0001*	0.46 0.0001*	0.19 0.017 ^s	0.26 0.001 ^s
TC	0.16 0.03 ^s	0.19 0.01 ^s	0.15 0.052	0.11 0.17	0.08 0.31
HDLc	-0.32 0.0001*	-0.13 0.09	-0.24 0.003 ^s *	-0.27 0.001	-0.31 0.0001*
HDL2	-0.36 0.0001*	-0.19 0.018 ^s	-0.28 0.0001*	-0.32 0.0001*	-0.36 0.0001*
HDL3	-0.05 0.51	0.04 0.58	-0.013 0.87	-0.005 0.95	-0.039 0.64
LDLc	0.26 0.001 ^s	0.14 0.06 ^s	0.28 0.001 ^s	0.083 0.31	0.15 0.05 ^s
sd-LDLc	0.37 0.0001*	0.31 0.0001*	0.37 0.0001*	0.24 0.003 ^s	0.31 0.0001*
hs-CRP	0.31 0.0001*	0.10 0.19	0.34 0.0001*	0.003 0.971	0.127 0.123
Fibrinogen	0.35 0.0001*	0.18 0.021	0.32 0.0001*	0.20 0.013 ^s	0.27 0.001 ^s

§ significant ; § very significant ; * Highly significant

- High sensitivity c-reactive protein (hs-CRP) (ELISA)

Anthropometric examination

- Waist circumference (WC)
- Hip circumference (HC)
- Waist to hip ratio (WHR)
- BMI

Observations were subjected to essential statistical analysis including mean \pm S.D., unpaired t-test, Pearson's coefficient of correlation and multiple regression analysis. Statistical analyses were done using Prism 5 software.

RESULTS

Obesity and hypertension were predominantly observed in PCOS women of present study (55%-BMI >25 kg/m²; 73%-WHR >80 cms; 84% had central adiposity; 62%-systolic and 67%-diastolic hypertension). Three or

Table 3: Multiple regression analyses of PCOS women

	Unstandardized coefficients B	SE	Standardized coefficients Beta	p value
Constant	4.367	0.744		0.000
Age	1.083E-02	0.005	0.129	0.025
BMI	1.528E-02	0.008	0.149	0.062
WC	-3.40E-03	0.004	-0.085	0.36
WHR	-3.31	0.471	-0.085	0.36
SBP	-7.778E-03	0.005	-1.41	0.15
DBP	-6.871E-03	0.007	-0.092	0.341
FBS	-8.136E-03	0.006	-0.384	0.191
BSPP	-4.119E-03	0.001	-0.256	0.356
HbA1C	2.008E-02	0.057	0.056	0.725
TG	-1.727E-04	0.001	-0.256	0.356
TC	1.685E-03	0.002	0.002	0.338
HDL	2.833E-02	0.194	0.432	0.884
HDL2	-1.140E-02	0.194	-0.145	0.953
HDL3	-3.809E-02	0.195	-0.280	0.087
sd LDLc	2.067E-03	0.003	0.077	0.54
hs-CRP	3.180E-02	0.195	-0.280	0.846
Fibrinogen	-3.901E-03	0.05	0.053	0.525
FSH	5.775E-02	0.023	0.173	0.014 ^s
LH	-4.744E-02	0.016	-0.219	0.005 ^s
Insulin	-2.187E-02	0.06	-0.062	0.842
HOMA-IR	0.174	0.053	1.269	0.001*

more than 3 components of MS (based on ATP III criteria) were seen in 66% of the PCOS women.

There was significant post-prandial hyperglycemia, hyperinsulinemia, IR, dyslipidemia, significant hypertriglyceridemia and reduced HDLc sub-fractions (HDL2c) and raised sd-LDLc in the PCOS women (Table 1). Further, there was significant increase in CVD risk ratios and fibrinogen levels. (Table 1) A significant association of anthropometric parameters with serum lipids, hs-CRP and fibrinogen was observed (Table 2). When data was analyzed with the help of multivariate regression analysis (Table 3), it showed age as an independent risk factor for PCOS. So, to get the independent effect of other risk factors besides age few other factors were found to be statistically significant with multivariate analysis, these were HOMA-IR, FSH, LH, which clearly indicated that HOMA-IR and FSH, LH vary in PCOS women independent of age and thus raise their risk of CVDs. Strongest cardiovascular risk factor assessed using multiple regression analysis was shown to be HOMA-IR, followed by FSH and LH indicated by their significant standardized beta coefficients (Table 3).

DISCUSSION

PCOS is a cluster of several traits common for CVD. Hyperandrogenism, a characteristic feature of PCOS

is associated with atherosclerosis and CVD risk in women.⁷ PCOS women have an abnormality of serine-threonine phosphorylation in insulin signalling⁸ and insulin increases ovarian androgen production, indicating a common aetiology for both IR and hyperandrogenism. Hyperinsulinemia produces a cluster of CVD risk factors including dyslipidemia, IGT, hypertriglyceridemia, sd LDLc and reduced HDLc, as observed in the present study.^{9,10} The presence of hyperinsulinemia, IR and MS can lead to cardiovascular complications in later life.¹¹

Based on ATP III criteria, the PCOS women fulfilling at least three criteria for MS (66%) stood a risk of CVD, since MS has been reported to be associated with worse in hospital outcomes and have a high risk of developing severe CVD.^{12,13} Further more hypertension, one of the leading cause of coronary artery disease (CAD) and cardiac failure¹⁴ was observed in PCOS women of present study.

Kinetic studies have shown obesity to be associated with hyperinsulinemia due to reduced metabolic clearance of insulin caused by diminished hepatic insulin extraction,¹⁵ as was suggested by significant correlation of WC and insulin in present study. (Table 2)

Hypertriglyceridemia has been reported as a strong risk factor of developing CAD, independent of other CAD risk factors across a broad population group within Asia-Pacific region.¹⁶ Furthermore, both amount and type of LDLc are significant in determining CAD risk. Sd-LDLc is more atherogenic, since it is more likely to enter the endothelium, get oxidised and undergo conformational changes, resulting in reduced receptor dependent clearance,

leading to atherosclerosis and CAD risk. A significant reduction of HDL2c (more cardio protective) in PCOS women of present study further raised CVD risk.¹⁷ Besides, markers of low grade chronic inflammation correlate well with CVD risk¹⁸ as indicated by raised fibrinogen and hs-CRP in PCOS women of present study.

PCOS is commonly associated with chronic, anovulatory infertility and hyperandrogenism with clinical manifestations of oligomenorrhea, hirsutism and acne.¹⁹ Recent reports show that PCOS was significantly associated with carotid intimal thickness and brachial artery flow mediated dilation (markers of sub-clinical atherosclerosis) independent of age, BMI and blood pressure.²⁰ Further in our study, the multivariate regression analysis showed that multiple factors like HOMA-IR, LH and FSH are independent risk factors of CVD risk for women with PCOS independent of the age. Besides there have been long term follow up studies reporting that there is no change in the CVD risk of post-menopausal women as compared to peri and premenopausal women.²¹ Evidences suggest ovarian hyperandrogenism is driven by abnormally high secretion of LH, acting on theca cells and that it is associated with atherosclerosis and CVD risk⁷ as suggested in present study by significant standardized beta coefficients of PCOS women.²²⁻²⁵ (Table 3)

CONCLUSION

Thus we recommend and conclude that in Indian PCOS women both conventional and novel CVD risk factors should be analysed. Obesity, IR, high pro-thrombotic and pro-inflammatory factors in PCOS indicated strong risk of CVD.

REFERENCES

- Allahbadia GN, Merchant R. Polycystic ovary syndrome in the Indian Sub continent. *Semin Reprod Med.* 2008; 26(1): 22-34.
- Knochenhauer ES, Key TJ, Kausar-Miller M, Waggoner W, Boots LR, Aziz R. Prevalence of the polycystic ovary syndrome in un selected black and white women of the south-eastern United States: a prospective study. *J Clin Endocrinol Metab.* 1998; 83(9): 3078-82.
- Rodin DA, Bano G, Bland JM, Taylor K, Nussery SS. Polycystic ovaries and associated metabolic abnormalities in Indian subcontinent Asian women. *Clin Endocrinol.* 1998; 49(1): 91-9.
- Cobin RH. Cardiovascular and metabolic risks associated with PCOS WOMEN. *Intern Emerg Med.* 2013; 81(1): S61-4.
- Azziz R, Maria C, Hoq L, Badamgarav E, Song P. Health care-related economic burden of the polycystic ovary syndrome during the reproductive life span. *J Clin Endocrinol Metab.* 2005; 90(8): 4650-58.
- Wijayaratrie CN, Balen AH, Barth JH, Belchetz PE. Clinical manifestations and insulin resistance (IR) in polycystic ovary syndrome (PCOS) among South Asians and Caucasians: is there a difference? *Clin Endocrinol.* 2002; 57(3): 343-50.
- Lett HS, Blumenthal JA, Babyak MA, Sherwood A, Strauman T, Robins C, *et al.* Depression as a risk factor for coronary artery disease: evidence, mechanisms, and treatment. *Psychosomatic Medicine A* 2004; 66(3): 305-15.
- Luque-Ramirez M, Mendieta-Azcona C, Alvarez-Balsco F, Escobar-Morreale HF. Androgen excess is associated with the increased carotid intima-media thickness observed in young women with polycystic ovary syndrome. *Hum Reprod.* 2007; 22(12): 3197-203.
- Sharma P, Gupta R, Purohit P. Cardiac Risk Factors in Descendants of Parents with History of Coronary Artery Disease (CAD) – An Evaluation Focusing on Small Dense Low-Density Lipoprotein-Cholesterol (sd LDL-C) and High-Density Lipoprotein cholesterol. *Ind J Biocm Biophys.* 2013. (accepted for publication)
- Frazier-Wood AC, Glasser S, Garvey WT, Kabagambe EK, Borecki IB, Tiwari HK, *et al.* A clustering analysis of lipoprotein diameters in the metabolic syndrome. *Lipids Health and Disease* 2011; 10: 237 doi:10.1186/1476-511X-10-237.
- Talbott EO, Zborowski JV, Rager JR, Boudreaux MY, Edmundowicz DA, Guzick DS. Evidence for an association between metabolic cardiovascular

- syndrome and coronary and aortic calcification among women with polycystic ovary syndrome. *J Clin Endocrinol Metabol.* 2004; 89(11): 5454-61.
12. Vega C and Barclay L. Medscape Medica news. May 24 2005.
13. Zeller M, Steg PG, Ravisy J, Laurent Y, Janin-Manificat L, L'Huillier I, Beer JC, Oudot *et al.* Observatoire des Infarctus de Côte-d'Or Survey Working Group. Prevalence and impact of metabolic syndrome on hospital outcomes in acute myocardial infarction. *Arch Intern Med.* 2005; 165(10): 1192-8.
14. Shroff R, Kerchner A, Maifeld M, Vanbeck E Jr, Jagasia D, Dokras A. Young obese women with polycystic ovary syndrome have evidence of early coronary atherosclerosis. *Clin Endocrinol Metab.* 2007; 92(12): 4609-14.
15. Orio F, Palomba S, Spinelli L, Cascella T, De Lorenzo A, Lucci R, *et al.* Cardio pulmonary impairment in young women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2004; 91(8): 2967-71.
16. Schannwell CM, Strauer BE. Hypertension and cardiac failure. *Internist (Berl).* 2007; 48(9): 909-20.
17. Lee SJ, Bacha F, Gungor N. Waist circumference is an independent predictor of insulin resistance in black and white youths *J paediatrics.* 2006; 148(2): 188-94.
18. Peiris AN, Sothmann MS, Hoffmann RG, Hennes MI, Wilson CR, Gustafson AB, *et al.* Adiposity, fat distribution, and cardiovascular risk. *Ann Intern Med.* 1989; 110(11): 867.
19. Ravi GR, Pradeepa R, Mohan V. Hypertriglyceridemia and coronary artery disease-an update. *Indian Heart J.* 2004; 56(1): 21-6.
20. Guleria AK, Syal SK, Kapoor A, Kumar S, Tiwari P, Dabadghao P. Cardiovascular disease risk in young Indian women with polycystic ovary syndrome. *Gynecological Endocrinology.* 2014; 30(1): 26-9.
21. Schmidt J, Landin-Wilhelmsen K, Mats Bra"nnstro"m, Dahlgren E. Cardiovascular Disease and Risk Factors in PCOS Women of Postmenopausal Age: A 21-Year Controlled Follow-Up Study. *J Clin Endocrinol Metab.* 2011; 96(12): 3794-803.
22. Morgan J, Carey C, Lincoff A, Capuzzi D. High-density lipoprotein sub fractions and risk of coronary artery disease. *Curr Atheroscler Rep.* 2004; 6(5): 359-65.
23. Dereli D, Ozgen G, Buyukkedeci F, Guney E, Yilmaz C. Platelet dysfunction in lean women with polycystic ovary syndrome and association with insulin sensitivity. *J Clin Endocrinol Metabol.* 2003; 88(5): 2263-68.
24. Legro RS, Urbanek M, Kunselman AR, Leiby BE, Dunaif A. Self-selected women with polycystic ovary syndrome are reproductively and metabolically abnormal and undertreated. *Fertil Steril.* 2002; 78(1): 51-7.
25. Topcu S, Caliskan M, Ozicmen EE, Tok D, Vckuyu A, Erdogan D, *et al.* Do young women with polycystic ovary syndrome show early evidence of preclinical coronary artery disease? *Human Reproduction.* 2006; 21(4): 930-5.
26. Sekharan PK. The infertility manual. Kamini A. Rao (Author, Editor), Peter R. Brinsden (Editor), A. Henry Sathananthan. Jaypee Publishers; 2005.