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High Sensitivity C – Reactive Protein and Insulin Resistance in Women with Polycystic Ovary Syndrome

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ABSTRACT

Background: *Polycystic Ovary Syndrome (PCOS) characterised by anovulation, polycystic ovaries and hyperandrogenism also leads to metabolic abnormalities such as insulin resistance (IR), deranged lipid profile, hypertension and a low-grade chronic inflammation. These factors are known to increase risk for cardiovascular diseases. This study aimed to compare various biochemical parameters between women with PCOS and age matched healthy controls.*

Methods*: Total 80 women diagnosed with PCOS were investigated for fasting plasma glucose, serum insulin, Insulin resistance (homeostasis model assessment, HOMA-IR), and hs-CRP and compared with 40 apparently healthy women. Cases of PCOS were divided in two groups based on BMI: PCOS with BMI 18.5 – 24.99 kg/m^2 and PCOS with BMI ≥ 25 kg/m^2.* ***Results****: Serum insulin levels and HOMA-IR values were significantly ($p<0.05$) higher in women with PCOS than controls. Levels of hs-CRP were also significantly higher in women with PCOS as compared to controls.*

Conclusions*: Early recognition of risk factors for cardiovascular disease in PCOS women is important from clinical and public health perspective.*

Key words*: PCOS, hs-CRP, HOMA-IR, cardiovascular disease.*

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INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of morbidity and mortality in the world. Atherosclerosis is the reason for the majority of CVD in these patients. It is accepted fact that inflammation plays a significant role in atherosclerosis.[1] PCOS is a proinflammatory state as is indicated by increase in the plasma concentrations of inflammatory mediators of atherogenesis like interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1), monocyte chemotactic protein-1 (MCP-1), C reactive protein (CRP), matrix metalloproteinase-2 (MMP-2) and plasminogen activator inhibitor-1 (PAI-1).[2] High sensitivity C- reactive protein (hs-CRP) has been shown to be a good predictor of vascular events.[3] hs-CRP is associated with features of metabolic syndrome as insulin resistance, abdominal obesity and dyslipidemia. Elevated hs-CRP in PCOS patients has been found to be associated with increased excess abdominal fat and IR.[1]

Therefore, this study was undertaken to compare the various biochemical parameters in women with PCOS and age-matched controls.

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METHODS

The study was conducted in Department of Biochemistry, Government Medical College, Aurangabad (Maharashtra),

India, with the prior approval of Institutional Ethical Committee. After a written informed consent, a total of 80 cases of PCOS were selected based on Rotterdam ESHRE/ASRM revised consensus on diagnostic criteria. According to Rotterdam consensus criteria commonly used in clinical practice, two of the following three must be fulfilled for the diagnosis of PCOS: polycystic ovaries, oligo-/anovulation clinically diagnosed as oligo-/amenorrhea and hyperandrogenism (clinical or biochemical).[4]

40 apparently healthy women in the age group of 18 to 35 years with BMI between 18.5 to 24.99 kg/m^2 were selected on the basis of history and clinical examination. They constituted the group 1 of the study. Cases of PCOS were divided in two groups based on BMI: Group 2- PCOS with BMI 18.5 – 24.99 kg/m^2 and Group 3- PCOS with BMI ≥ 25 kg/m^2.

Detailed history of participants including age, marital status, history of any medications, addictions was taken. Known cases of Type 1 or Type 2 DM, history of alcoholism, cardiovascular disease, renal diseases, any endocrinological disorders, medications that increase body weight (contraceptive pills, steroids), patients on statin therapy, pancreatic disorders were excluded.

After overnight fasting, venous blood samples were collected in plain and fluoride bulbs. Plasma glucose was measured using glucose-oxidase-peroxidase method. Quantitative estimation of serum insulin and hs-CRP was done by Chemiluminescence Immunoassay (CLIA) using Acculite CLIA microwells. Assay kits from Monobind INC., Lake Forest, CA 92630, USA.

Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR) with the formula:[5]

$$\text{HOMA IR Index} = \frac{\text{Fasting Blood Glucose (mg/dl)} \times \text{Fasting Serum Insulin (µIU/ml)}}{405}$$

The results were analyzed by Graph pad prism software, version 5. The results were interpreted as mean ± S.D. Unpaired t test was applied for comparing between the groups and correlation coefficients were calculated (r value). P value was obtained from unpaired t test and < 0.05 was considered statistically significant. Correlation coefficients (r) were calculated among various parameters in group 2 and group 3. Positive and negative r values were interpreted as follows: r: 0 (no correlation), r: 0- 0.3 (poor correlation), r: >0.3- 0.7 (considerable correlation) and r: 0.8 or more (strong correlation).

RESULTS

Table 1 shows that values of the demographic characters in Group 1 and group 2 did not differ significantly among the groups. Women in Group 2 and group 3 showed highly significant difference in the mean values of weight, BMI, waist circumference(WC) and waist hip (W/H) ratios (p2 < 0.0001). Also the mean values of hip circumference (HC) (p2= 0.04) differed significantly among the two groups.

Table 2 shows that mean values of insulin, HOMA-IR and hs-CRP are significantly increased in group 2 as compared to their matched controls (group 1). The mean values of insulin, HOMA-IR and hs-CRP were significantly higher in group 3as compared to group 2. Threshold point for defining insulin resistance by HOMA IR Index was taken as 2.5.

We can see from Table 3 that in the lean PCOS patients, hs-CRP shows a significant correlation with BMI, waist/hip ratio, insulin and HOMA-IR. It is poorly correlated with BSL. Insulin shows a significant correlation with all parameters but is poorly co-related with BMI. HOMA-IR is significantly correlated with all parameters.

Table 4 shows that in overweight and obese PCOS i.e. group 3, Hs-CRP shows a positive correlation with BMI, waist/hip ratio, insulin, HOMA-IR. It is poorly correlated with BSL. Insulin shows a positive correlation with all parameters. HOMA-IR is positively correlated with all parameters.

DISCUSSION

Stein and Leventhal first described PCOS as a symptom complex consisting of amenorrhoea, hirsutism and enlarged polycystic ovaries. However, numerous researches in the last few years have demonstrated that, in addition to the effects on female reproductive system, PCOS also has metabolic and cardiovascular implications.[6]

Insulin resistance occurs in around 50% to 80% of women with PCOS.[7,8] Obesity occurs in about 60% of women with PCOS. The central distribution of fat is found to be associated with higher insulin concentrations. Hyperinsulinemia appears to play a main role in increasing the cardiovascular risk in women with PCOS.[9] Insulin resistance in vascular tissue is related with endothelial dysfunction, decreased arterial compliance, and possible development of CVD in patients with PCOS.[10]

Polycystic Ovary Syndrome (PCOS) is a proinflammatory state.[11] Evidence for the presence of cardiovascular risk factors such as inflammation, oxidative stress and impaired fibrinolysis has also been found in women with PCOS.[7] The chronic low level inflammation in women with PCOS can escalate the risk of atherogenesis. Elevations in a number of circulating proatherogenic inflammatory mediators such as interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1), monocyte chemotactic protein-1 (MCP-1), C-reactive protein (CRP), matrix metalloproteinase-2 (MMP-2) and plasminogen activator inhibitor-1 (PAI-1) have all been independently reported in the disorder.[2]

According to American Heart Association (AHA) guidelines 2002, CRP [as measured by high sensitivity assay (hs-CRP)] is the inflammatory marker of choice, as it has most stability, assay precision, accuracy and availability.[12] Serum high-sensitivity C-reactive protein (hs-CRP) is an independent cardiovascular risk factor. Serum concentrations of hs-CRP, even within the normal range, independently predict myocardial infarction and ischemic stroke. Hs-CRP levels

Table 1: Comparison of demographic Characters in studied Groups:

Parameter	Group 1 Healthy women with BMI 18.5- 24.99 n = 40	Group 2 PCOS with BMI 18.5-24.99 n = 40	p1 value	Group 3 PCOS with BMI ≥25 n = 40	p2 value
	MEAN ± SD			MEAN ± SD	
Age (Years)	25.63±2.38	25.58± 3.90	0.94	27.08 ± 3.94	0.09
Weight(Kg)	57.25± 5.54	57.27± 5.86	0.98	77.75 ± 7.26	**<0.0001****
Height(m)	1.59 ± 0.05	1.58 ± 0.06	0.60	1.60 ± 0.04	0.20
BMI(Kg/M²)	22.65± 1.19	22.86± 1.30	0.45	30.48 ± 2.50	**<0.0001****
WC (Cm)	76.83± 3.15	78.68± 5.15	0.06	87.85 ± 6.17	**<0.0001****
HC (Cm)	95.70± 5.16	96.06± 5.93	0.75	98.63 ± 5.08	**0.04***
W/H Ratio	0.80 ± 0.04	0.82 ± 0.04	0.09	0.89 ± 0.07	**<0.0001****

(p1 value: p value for group 1 & 2; p2 value: p value for group 2 & 3)
* Significant p value
** Highly significant p value

Table 2: Comparison of BSL, serum insulin, HOMA-IR & hs-CRP in studied groups

Parameter	Group 1 healthy women with BMI 18.5- 24.99	Group 2 PCOS with BMI 18.5-24.99	p1 value	Group 3 PCOS with BMI ≥25	p2 value
	MEAN ±SD			MEAN ±SD	
Glucose (mg %)	92.28 ± 8.33	96.05 ± 9.83	0.07	97.33 ± 7.48	0.52
Insulin (µIU/ml)	5.71 ± 1.79	9.38 ± 2.58	**<0.0001****	11.52 ± 2.30	**0.0002****
HOMA-IR Index (< 2.5)	1.28 ± 0.36	2.26 ± 0.74	**<0.0001****	2.79 ± 0.67	**0.001****
hs-CRP(upto 1 mg/L)	0.83 ± 0.17	1.63 ± 0.38	**<0.0001****	2.79 ± 0.53	**<0.0001****

have been linked to insulin resistance and are also associated with the metabolic syndrome which increases the risk of atherosclerosis.[13] CRP may directly promote endothelial dysfunction by increasing synthesis of soluble adhesion molecules and the secretion of monocyte chemoattractant protein and also by facilitating macrophage LDL uptake.[14] Obesity occurs in about 60% of women with PCOS.[9]

Table 3: Correlation coefficients (r value) in Group 2: PCOS BMI 18.5-24.99

	BSL	Insulin	HOMA-IR	hs-CRP
BMI	0.34 <0.05	0.26 0.1	0.32 <0.05	0.33 <0.05
W/H	0.37 <0.05	0.36 <0.05	0.40 <0.05	0.38 <0.05
Insulin	0.55 **<0.001**	-	0.97 **<0.001**	0.42 **<0.01**
HOMA-IR	0.73 **<0.0001**		-	0.38 <0.05
hs-CRP	0.17 0.28			-

Obesity, a proinflammatory state, is known to be independently associated with elevations in inflammatory markers. The inflamed adipose tissue is a source of IL-6 and TNF-α. IL-6 stimulates CRP synthesis in the liver. The degree of elevation in circulating levels of CRP and IL-6 in PCOS is much greater when obesity is also present,[11] although, elevated circulating CRP independent of obesity, is evident in both obese and nonobese women with PCOS.[10] In the present study it was found that that the serum insulin and hs-CRP levels were increased in both lean PCOS group and overweight and obese PCOS group, with a greater rise in the latter group. Also the serum insulin and hs-CRP levels correlated strongly with waist/hip ratio. This suggests role of abdominal obesity (waist/ hip ratio) on serum insulin and hs-CRP.

Table 4: Correlation coefficients (r value) in group 3: PCOS with BMI >25

	BSL	Insulin	HOMA-IR	hs-CRP
BMI	0.25 0.2	0.44 **<0.01**	0.45 **<0.01**	0.30 0.06
W/H	0.43 **<0.01**	0.55 **<0.001**	0.61 **<0.0001**	0.35 <0.05
Insulin	0.40 <0.05	-	0.96 **<0.0001**	0.41 **<0.01**
HOMA-IR	0.63 **<0.0001**		-	0.38 <0.05
hs-CRP	0.12 0.45			-

The potential mechanism that may explain significant correlation of serum insulin and hs-CRP with waist/ hip ratio may be as follows:

1) The anatomical proximity of visceral fat to the portal venous system leads to the direct drainage of metabolites and secretory products like free fatty acids (FFA) to the liver resulting in hepatic insulin resistance which in turn may lead to increased hepatic gluconeogenesis.[15]

2) Visceral adipocytes are lipolytically more active than subcutaneous adipocytes which could make visceral fat deposition more deleterious than subcutaneous abdominal fat.[15]
3) Visceral adipocytes produce proinflammatory cytokines like TNF-α, IL-1 and IL-6, which disrupt normal insulin action in fat and muscle cells and may be a major factor in causing whole body insulin resistance.[16]
4) Presence of visceral obesity could lead to increased production of cytokines such as IL-6 and TNF-α by visceral adipocytes, which could induce higher hs-CRP production by liver.[11]

Ahmed M. Mohamadin et al, in their study found that women with PCOS had significantly higher fasting serum insulin levels and hs-CRP compared to healthy controls ($p < 0.001$).[3] Boulman et al found that women with PCOS with normal BMI and obese women with PCOS had raised hs-CRP as compared to matched controls ($p < 0.001$).[17] Orio et al found that women with PCOS had significantly higher levels of CRP, glucose, insulin and HOMA-IR as compared to controls ($P < 0.0001$).[18] In a study by Fatma Ferda Verit, it was found that PCOS patients had increased hs-CRP compared to the control group ($p < 0.0001$). Hs-CRP was positively correlated with body mass index (BMI) ($r = 0.44$, $p < 0.0001$) and waist-to-hip ratio (WHR) ($r = 0.66$, $p < 0.0001$).[1]

The results of present study indicate that there was a positive correlation between serum insulin levels and serum hs-CRP in both lean PCOS (group 2) and overweight and obese PCOS (group 3) suggesting the role of acute phase reactants in development of insulin resistance.

The probable mechanisms influencing role of acute phase reactants such as hs-CRP on insulin resistance may be following:

1) The visceral adipocytes play key role in regulating inflammation. CRP synthesis in the liver is regulated by proinflammatory cytokines like IL-6 and TNF-α released by adipocytes. This suggests that, association of increased hs-CRP concentration with increased insulin levels could be due to presence of chronic systemic sub-clinical inflammation.
2) CRP bound to membranes of damaged vascular cells activates complement proteins or enhances the production of thrombogenic agents. This vascular inflammation may contribute to the development of insulin resistance.[19]

In a study Tarkun et al compared nonobese cases of PCOS with age and weight matched controls. They found that levels of fasting insulin and hs-CRP were increased significantly in cases as compared to controls ($p < 0.05$ and 0.007 respectively). Also the levels of hs-CRP correlated with BMI ($r = 0.360$, $p = 0.006$), fasting insulin ($r = 0.257$, $p = 0.06$) and HOMA ($r = 0.270$, $p = 0.04$).[14] Flavia Tosi et al found that hs-CRP concentrations were higher in PCOS women as compared to lean controls ($p < 0.05$). They also found significant positive correlation of serum hs-CRP with BMI ($r = 0.26$, $p = 0.037$), and negative correlation with insulin mediated glucose uptake ($r = -0.40$, $p = 0.001$), thus concluding that body fat and insulin resistance independently determined the chronic inflammation seen in PCOS.[13]

In the present study, we also found significant correlation of serum hs-CRP levels with HOMA-IR values. Gonzlez et al in their study found that CRP levels showed a positive correlation with BMI, total body fat (%), truncal fat(%), waist circumference and HOMA-IR ($p < 0.05$ for all). They concluded that both PCOS and obesity contribute to a proatherogenic state in women with PCOS.[2] Ritu Karoli *et al* in their study to assess atherosclerotic risk factors in women with PCOS found that hs-CRP was correlated with BMI ($r = 0.54$, $p = 0.005$) and HOMA ($r = 0.38$, $p = 0.02$).[20] Ji Young Oh et al found higher levels of hs-CRP in lean women with PCOS as compared to controls ($p < 0.05$). hs-CRP levels were correlated with waist circumference ($r = 0.46$, $p < 0.01$), BMI ($r = 0.46$, $p < 0.01$) and were negatively associated with insulin-mediated glucose uptake (IMGU) ($r = -0.31$, $p = 0.07$).[21] Kelly et al in their study found that women with PCOS had significantly elevated hs-CRP concentrations relative to controls ($P = 0.016$). Log CRP correlated with BMI ($r = 0.58$, $p < 0.05$) and inversely with insulin sensitivity ($r = -0.57$, $p < 0.05$). On adjustment for insulin sensitivity, log CRP was no longer significantly different between the groups ($p = 0.14$). They proposed low grade chronic inflammation as a novel mechanism for increased CVD risk in PCOS.[22]

CONCLUSION

From the findings of present study we can conclude that, correlation between insulin resistance and hs-CRP levels increase the risk for cardiovascular diseases in women with PCOS. Early identification of these risk factors is necessary to improve the quality of life in women with PCOS.

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