EVALUATION OF THE HIGH-SENSITIVITY C-REACTIVE PROTEIN IN YOUNG OBESE WOMEN WITH PCOS

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ABSTRACT

BACKGROUND
The Polycystic Ovarian Syndrome (PCOS) is one of the most common reproductive disorder in young women affecting 5-10% of population. PCOS women are at increased risk of developing metabolic syndrome, type 2 diabetes mellitus and cardiovascular diseases. PCOS is now recognised as not only a reproductive disorder, but also a metabolic one with long-term effects on women's health. With this background, the present study was undertaken to assess the levels of High-Sensitivity C-Reactive Protein (hs-CRP) in young obese women with PCOS as compared with healthy obese women without PCOS.

MATERIALS AND METHODS
This cross-sectional observational study was carried out in MKCG Medical College, Berhampur, in the Department of Cardiology and Gynaecology between January 2016 to December 2016. A total of 56 young obese PCOS patients aged less than 30 years and 25 healthy patients matched for age and BMI were studied.

RESULTS
Baseline cardiovascular risk factors, hormone variables and lipid profiles and hs-CRP levels are measured in both PCOS patients and control subjects. It has been observed that the median hs-CRP levels are significantly higher in young obese PCOS patients than the control subjects. Obese patients with PCOS had higher levels of hs-CRP compared to healthy obese controls. The mean values of hs-CRP was 5.46 mg/L in PCOS group and 2.8 mg/L in the control group, which is statistically significant.

CONCLUSION
PCOS patients clearly present a higher risk of CVD due to its peculiar hormonal pattern characterised by insulin resistance, dyslipidaemia and inflammatory state. The metabolic disorders in PCOS could possibly be improved by diet and drugs in early periods of their life, so as to decrease the risk of CVD in future. Estimation hs-CRP maybe considered as a reliable predictive marker for future Cardiovascular Disease (CVD) in PCOS patients.

KEYWORDS
PCOS, Hs-CRP, Obesity, CVD.

BACKGROUND
Polycystic ovarian syndrome affects about 5-10% of women in reproductive age group.[1]

PCOS is now recognised as not only a reproductive disorder, but also a metabolic one with long-term effects on women's health. It has been found to correlate with traditional cardiovascular risk factors such as increasing age, obesity and adverse lipid profiles.[2] Clinically, PCOS can be diagnosed in women having hirsutism, irregular menstrual cycle and classical ovarian morphology.[3] Limited data are available concerning the prevalence of metabolic disorders in young women with PCOS and whether chronic PCOS

status overtime will lead to greater impairment of cardiovascular status is still to be evaluated. It has been precisely identified that insulin resistance and hyperinsulinaemia are two triggering conditions for onset of PCOS.

The pathophysiology of PCOS is considered multifactorial involving genetic, environmental and metabolic abnormalities. Among the genetic causes, recently it has been found that there is mutation in genes involved in the synthesis, transport and regulation of androgen. However, as several genes are simultaneously involved in the pathogenesis of PCOS, the genetic basis is yet to be understood completely. Suggested exogenous factors responsible for PCOS are diet, physical activity and lifestyle, which may influence the phenotypic manifestation of the syndrome. Regarding the metabolic abnormalities, it has been seen that there occurs a proinflammatory state caused by oxidative stress, which is associated with insulin resistance, hyperandrogenism and PCOS. PCOS is often associated with two relevant morbidities like diabetes and obesity. It has been seen that childhood obesity is a well-documented risk factor for obesity. Similarly, women with

PCOS are at increased risk of having obesity. Increased androgen levels have been found in women with upper body obesity with increased visceral/subcutaneous fat, which directly correlates with the degree of insulin resistance.[4] In several epidemiological studies, it has demonstrated that hs-CRP, a marker of inflammation is a strong predictor of CVD. Both clinical and laboratory data confirms that atherosclerosis is a disease of both lipid accumulation and a state of chronic inflammation.[5,6] Individuals with LDL-C level less than 130 mg/dL along with CRP level more than 3 mg/L represent a high-risk group for CVD. Therefore, in view of possible ability of CRP in predicting cardiovascular morbidity and mortality in apparently healthy individuals, it may be used as an ideal marker for screening of healthy young PCOS patients.

The present study was setup to evaluate the presence of marker of systemic inflammation (hs-CRP) in young women with PCOS. PCOS is often associated with obesity, mainly abdominal obesity and insulin resistance. Visceral fat is the most significant variable correlating with metabolic dysfunction in women with PCOS. Visceral adipose tissue secretes various adipokines and vasoactive substances associated with an increased risk of metabolic and cardiovascular diseases. In an attempt to predict cardiovascular risk, efforts have been focused on hs-CRP- a marker of low-grade subclinical inflammation.[7,8] Increased levels of this protein are associated with abdominal obesity, DM and CVD. Several large scale prospective epidemiological studies have demonstrated that hs-CRP is a strong independent predictor of future CVD and/or stroke. It is evident that atherosclerosis in addition to being a disease of lipid accumulation is also a chronic inflammatory process. It is thus possible to identify those PCOS patients who are at increased risk of metabolic syndrome basing on their hs-CRP levels.

MATERIALS AND METHODS

The study was conducted in the OPD of Department of Obstetrics and Gynaecology and Cardiology in MKCG Medical College and Hospital, Berhampur, between January 2016 to December 2016. This study was conducted to compare the levels of hs-CRP in young obese patients with PCOS (study group) and healthy female patients matched for age and body mass index, but without PCOS (control group). PCOS was diagnosed using the Rotterdam criteria for PCOS (presence of two out of three- oligoovulation or anovulation, clinical and/or biochemical signs of hyperandrogenism or polycystic ovaries). Patients without a history of diabetes, dyslipidaemia, hypertension or cardiovascular diseases were included in this study. Clinical and anthropometric measurements like age, weight, waist-hip ratio, BMI and

blood pressure were recorded. Laboratory and radiological investigations like FBS, total cholesterol, HDL, LDL, triglycerides, hs-CRP, fasting insulin and USG of abdomen and pelvis were evaluated. Hs-CRP was analysed by latex-enhanced turbidimetric assay. Hs-CRP is divided into three categories according to risk stratification for CVD. Less than 1 mg/L as low risk, 1-3 mg/L as intermediate risk and 3-10 mg/L as high risk. Levels >10 mg/L is associated with active infection and hence were excluded from the study.

Statistical Analysis- Statistical analysis was carried out using the SPSS-PC 13.0 (SPSS Inc., Chicago, IL, USA). Distribution of variables was tested with Kolmogorov-Smirnov test. Results were reported as means ± Standard Deviation (SD) for continuous variables with normal distribution, median (1st and 3rd) quartile for continuous variables with a distribution that differs from normal distribution and % for dichotomous data. T-test and Mann-Whitney tests (as appropriate) were used to compare data. According to variables distribution, Pearson or Spearman correlation coefficient was calculated. General linear model was used to adjust the mean value of the hs-CRP levels for BMI. The level of significance was set at 5% ($p<0.05$) in all analyses.

RESULTS

A total of 56 PCOS patients and 25 control subjects were studied. Anthropometric and metabolic characteristics of the patients included in the study are presented in Table 1. The mean age of the PCOS group was 26.5 ± 8 years and the mean age of the control group was 29.2 ± 8 years. There were no anthropometric or biochemical parameter differences between the study group and control group.

The comparison of mean age and BMI between the PCOS and control groups is depicted in Table 2. In this study, BMI >25 kg/m^2 was considered as obese, both in study and control groups as per the "consensus statement for diagnosis of obesity and metabolic syndrome for Asian Indians" by Mishra et al. Patients were categorised according to the presence or absence of metabolic syndrome. Metabolic syndrome was present in 39% young obese PCOS patients, however, only 16% of non-PCOS obese controls had metabolic syndrome (Table 3). The hs-CRP levels are presented Table 4. Hs-CRP value was more than 3 mg/L in 39% of PCOS patients, whereas only 8% of non-PCOS controls had an equivalent value. Mean hs-CRP level was 5.4 mg/L in PCOS patients with metabolic syndrome, and in non-PCOS controls with metabolic syndrome, it was 2.8 mg/L ($p<0.001$). Using univariate analysis, hs-CRP levels were significantly correlated with BMI in PCOS group, but not in control group (Table 5).

Sl. No.	Parameters	PCOS Group (n=56)	Control Group (n=25)	p value
1.	Age (in years)	26.5	29.2	0.4
2.	BMI	31.4	30.8	0.1
3.	Waist-hip circumference (in cm)	86.4	84.5	0.6
4.	FBS (mg/dL)	104	94	<0.01
5.	Total cholesterol (mg/dL)	168	148	<0.001
6.	HDL (mg/dL)	38.8	48.6	<0.001

7.	LDL (mg/dL)	134	94	0.05
8.	Triglyceride (mg/dL)	140.8	122.6	<0.1
9.	Blood pressure (mmHg)	144/90	124/86	0.32
	Table 1. Anthropometric and Metabolic Characteristics			

Parameters	PCOS Group (n=56)	Control Group (n=25)	p value
Age in years	26.5 ± 8	29.2 ± 8	0.01
BMI (kg/m^2)			
25-30	35 (62%)	18 (72%)	0.050
>30	21 (38%)	7 (28%)	0.065

Parameters	PCOS Group (n=56)	Control Group (n=25)	p value
Metabolic syndrome present	23 (39%)	4 (16%)	<0.001
Metabolic syndrome absent	33 (64%)	21 (84%)	<0.1
Table 3. Prevalence of Metabolic Syndrome			

Hs-CRP Levels	PCOS Group (n=56)	Control Group (n=25)	p value
<1 mg/L	8 (14%)	12 (48%)	<0.5
1-3 mg/L	26 (46%)	11 (44%)	<0.001
>3 mg/L	22 (39%)	2 (8%)	<0.001
Table 4. Hs-CRP Levels in PCOS and Control Groups			

	Mean hs-CRP Level (mg/L)	P value
PCOS with metabolic syndrome, n=23	5.4	<0.001
PCOS without metabolic syndrome, n=33	2.1	<0.01
Non-PCOS + Met syndrome, n=4	2.8	<0.001
Non-PCOS - Met syndrome, n=21	1.8	<0.01
Table 5. Hs-CRP Levels in PCOS with and without Metabolic Syndrome		

DISCUSSION

Women with PCOS are at increased risk of developing cardiovascular diseases. These women may also have an increased prevalence of subclinical atherosclerotic disease as suggested by greater carotid intimae media thickness. Dahlgen et al have calculated using a risk model analysis that PCOS patients had a 4-7 fold higher risk of myocardial infarction compared with age-matched controls.[9] Birdsall et al have studied the association between PCOS and coronary artery disease in 143 women aged 60 years or younger undergoing cardiac catheterisation. PCOS was detected in 42% of patients. By multivariate regression analysis,[10] the extent and severity of coronary artery disease have been independently associated with PCOS (p=0.023). Although, the association between PCOS and CVD has been repeatedly suggested by several reports. It has not been unequivocally substantiated. The traditional cardiovascular risk factors like hypertension, dyslipidaemia, obesity and insulin resistance are aggravated in PCOS. Women with PCOS are at increased risk of developing hypertension in later life. The probable mechanisms are yet to be delineated. Insulin resistance causes secondary hyperinsulinaemia. Hyperinsulinaemia may produce enhanced sodium retention resulting in increased intracellular sodium and calcium and augmentation of sympathetic activity, which may have a role in development of hypertension. Again, hyperandrogenaemia in PCOS plays an important role in development of hypertension. Androgen may directly stimulate Endothelin-1 (ET-1) or may stimulate the renin-angiotensin system to increase ET-1, thus leading to the expression of two powerful vasoconstrictors.

The altered lipid profile pattern (increased TG, low HDL and increased small dense LDL subclasses is similar to that seen in diabetes mellitus. It is mainly the consequence of insulin resistance that impairs the ability of insulin to suppress lipolysis thereby increasing mobilisation of FFA from adipose stores. Consequently, increased hepatic delivery of FFAs impairs insulin inhibition of hepatic VLDL synthesis. Additionally, the ability of insulin resistance to alter the expression of lipoprotein lipase and hepatic lipase may also contribute to this lipid pattern. Due to this fact, adipose tissue increases insulin resistance and this pattern is likely to be found in obese patients with PCOS.

Accumulation of visceral fat leads to insulin resistance, endothelial dysfunction and proinflammatory status through fat derived metabolic products, hormones (adiponectin, resistin, FFA) and cytokines (IL-1, IL-6, IL-18, TNF-α).[11] Obesity and PCOS interact to promote premature atherosclerosis and CV mortality.[12] Insulin Resistance (IR) occurs in 50-80% of women with PCOS. Mechanism involved in IR are likely to be complex with genetic and environmental contribution Specific abnormalities of insulin metabolism identified in PCOS includes reduction in secretion of insulin, reduced hepatic extraction, impaired suppression of hepatic neoglucogenesis and abnormalities in the insulin receptor signalling. But, paradoxically in PCOS, there is IR where insulin stimulated androgen production persists, while its role in glucose metabolism is impaired. Thus, IR in PCOS results in hyperinsulinaemia with its diverse and complex effects on regulating lipid metabolism, protein synthesis and modulation of androgen production. PCOS patients with IGT have a fivefold risk of developing type-2 DM and CVD over 8 years (Wild et al, 2010).[13]

Apart from these traditional cardiovascular risk factors, there is certain non-traditional cardiovascular risk factor. They are adiponectin, leptin, hs-CRP, IL-6 and Plasminogen Activator Inhibitor-1 (PAI-1).[14] They were associated with accelerated atherosclerosis in diabetes mellitus and might also be involved in the pathogenesis of vascular disease in PCOS. Platelet function abnormalities, alterations in coagulation cascade or a prothrombotic state can be seen with diabetes and hyperinsulinaemia. Elevated concentration of circulating homocysteine has been identified as another cardiovascular risk factors causing endothelial oxidative stress and platelet aggregation and consequently leading to

atherosclerosis. Significantly elevated concentrations of homocysteine have been documented in both lean and obese PCOS. Adiponectin, a crucial adipocytokine has been shown to inhibit endothelial inflammation stimulates the production of nitric oxide in the endothelium and protects the blood vessel from damage associated with insulin resistance. It has been shown in many studies that there is decreased levels of adiponectin in women with PCOS.

Recently, it has been emphasised that there occurs a state of low-grade chronic inflammation probably due to insulin resistance, hyperandrogenaemia and abdominal adiposity in PCOS patients. Low-grade chronic inflammation can be defined as a condition characterised by increased circulating levels of several mediators of inflammation triggered by TNF-α, IL-1, IL-6 and hs-CRP. Visceral adipocytes are able to produce these cytokines that act directly or indirectly as mediators of systemic inflammation. Chronic inflammatory state with obesity is related to the insulin resistance state and PCOS. Atherosclerosis represents a state of chronic inflammatory process.

Hs-CRP is a widely studied marker of low-grade inflammation, which can significantly predict CVD. Hs-CRP is not only an inflammatory marker of atherosclerosis, but also have some direct deleterious effects on the vascular wall contributing to the pathogenesis of lesion formation, plaque rupture and coronary thrombosis by interacting with endothelial cell phenotype. Hs-CRP directly influences the endothelium derived vasoactive factors and decreases the production of nitric oxide. Shroff et al have demonstrated increased levels of hs-CRP in women with PCOS. However, many studies have demonstrated that women with PCOS the hs-CRP values are primarily dependent upon coexistent obesity and insulin resistance. Kelly et al in their study shown that elevated CRP levels are found in obese and non-obese PCOS patients compared to controls. Tarkum et al cited significantly higher CRP values in obese than in non-obese PCOS subjects.[15] Boulman et al in their study found that 36.8% of the PCOS patients had hs-CRP levels above 5 mg/L, whereas only 9.6% of the controls exhibited such high hs-CRP levels (p<0.001).[2] In our study, metabolic syndrome was present in 36% of obese PCOS patients, but only 8% in the control group had metabolic syndrome. The mean hs-CRP level was 5.46 mg/L in PCOS patients and it is only 2.8 mg/L in non-PCOS control subjects (p<0.001).

CONCLUSION

PCOS is associated with a low-grade inflammation mainly due to accumulation of visceral fat, insulin resistance and hyperandrogenaemia. A vicious cycle occurs with continuous release of inflammatory mediators that are responsible for the development of IR, dyslipidaemia, endothelial dysfunction and long-term metabolic and cardiovascular complications. The hypertrophic adipocytes secrete different inflammatory markers and adipokines, which in turn determine the development of IR and an increase of the hepatic production of hs-CRP. It also produces increased production of ovarian, adrenal androgen production, which may aggravate central adiposity distribution and vascular

injury. Hs-CRP level measurement is readily available and simple measurement, which can be considered as a possible risk factor analysis method to predict future cardiovascular risk assessment in PCOS subjects. Metabolic disorders could possibly improve by offering treatment such as diet, cessation of smoking, lifestyle modification measures, exercise and drugs like metformin, aspirin, antihypertensive drugs and statins. These measures may reflect in lowering of the hs-CRP levels thereby decreasing the risk of future cardiovascular events.

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