ORIGINAL RESEARCH PAPER

Biochemistry

KEY WORDS: Dyslipidemia, hs CRP, Polycystic Ovary Syndrome.

STUDY OF ASSOCIATION OF HIGHLY SENSITIVE C-REACTIVE PROTEIN WITH LIPID PROFILE IN POLYCYSTIC OVARY SYNDROME PATIENTS WITHOUT INSULIN RESISTANCE IN A TERTIARY CARE CENTRE KOLKATA

Chinmoy Ghosh Assistant Professor, Department Of Biochemistry, NRS Medical College, Kolkata.

Santasmita Pal* Assistant Professor, Department Of Biochemistry, Medical College, Kolkata. *Corresponding Author

ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a common endocrinopathy involving ovulatory disturbances, hyperandrogenism and infertility.The goal of the study was to compare cardiovascular heart disease risk factors in women with polycystic ovary syndrome (PCOS) and matched control subjects.

Aims and objectives:The present study aims to investigate highly sensitive serum C-reactive protein (hs CRP) levels in normoinsulinemic polycystic ovary syndrome (PCOS) patients and whether there was any relationship between HS-CRP and other cardiovascular risk factors such as serum lipid profile.

Materials and methods:The study included 150 PCOS patients without Insulin resistance from Gynaecology OPD of a tertiary care hospital of Kolkata,West Bengal, and 150 age- matched healthy volunteers. Standard clinical examinations, and ultrasonographic and endocrine screening including FSH, LH, total testosterone, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC) and triglyceride (TG) and hs CRP were measured.

Results: Women with PCOS had significantly increased cardiovascular disease risk factors compared with control women like increased total cholesterol, LDL cholesterol, and triglyceride levels ($P<0.001$), decreased total HDL levels ($P<0.001$), and increased hs CRP levels. ($P<0.05$).These risk factors were especially elevated in PCOS women in the early reproductive age compared with control women, indicating that women with PCOS should be monitored for early detection of cardiovascular risk and considered for appropriate clinical interventions.

Conclusion: Though this is a small study, it has highlighted the association of dyslipidemia and increased hs CRP level even in normoinsulinemic PCOS subjects. Deranged lipid profile and pro-inflammatory factors in Indian PCOS women indicate strong CVD risk and could cause cardiovascular complications in later life.

INTRODUCTION

Polycystic ovary syndrome is the most common endocrinopathy among women.[1]It has been reported to have a prevalence of 6-12%.[2] The present diagnostic criteria of this heterogenous disorder, according to the Androgen Excess & PCOS Society (AE-PCOS society) are: hyperandrogenism (clinical &/ or biochemical), ovarian dysfunction (ovulation disturbance&/ or polycystic ovary morphology) and the exclusion of other causes of androgen excess or related disorders. [3] Increasing evidence has emerged for association of increased cardiovascular risk compared with age matched controls in patients with polycystic ovary syndrome (PCOS) has. It has been estimated that myocardial infarction is seven times more likely in patients with PCOS [4] and cardiac catheterisation studies have shown more extensive coronary artery disease in these patients than in women with normal ovaries. [5] This increased cardiovascular risk is probably in part, attributed to metabolic disturbances like dyslipidemia, diabetes, and obesity that tend to cluster in women with PCOS. However it is not known whether the increased cardiovascular risk seen in PCOS is mediated through obesity per se or is independent of body mass index (BMI) and the result of other metabolic factors.

In recent years, interest has grown in novel biochemical and biophysical markers of cardiovascular risk. Highly sensitive C reactive protein (hs CRP) has been shown to be a good predictor of vascular events. In addition to being a marker of inflammation, there is evidence that HS CRP may have a direct role in atherogenesis via adhesion molecule expression, complement activation, and mediation of low density lipoprotein (LDL) uptake by macrophages. [6]. Increased HS-CRP levels have been reported in PCOS patients favouring the hypothesis that PCOS increases cardiovascular risk by activating chronic inflammation [7], although other authors reported that inflammatory markers in PCOS patients were not increased when compared with age and BMI matched controls. [8,9]

The aim of the present study was to evaluate the circulating concentrations of hs CRP as serum markers of inflammation in a group of premenopausal women with PCOS compared with healthy controls,focusing on their relationship to dyslipidemia.

MATERIALS AND METHODS

Study setting: *The* study was conducted in the Department of Biochemistry, Medical College, Kolkata from January 2019 to January 2020.

***Informed Consent*:** Written informed consent was taken from the patients as per Proforma . Demographical data, detailed history and clinical findings and laboratory investigations were recorded in the Proforma.

Ethical Clearance: This study was cleared by Institutional Ethics committee.

Study group: Study group patients are those suffering from polycystic ovary syndrome as diagnosed by criteria laid down by Androgen Excess and PCOS Society (AE-PCOS Society), attending Gynaecology OPD of Medical College and Hospital, Kolkata.

Control group: Age matched healthy subjects not suffering from PCOS and anovulation, attending OPD Biochemistry Lab. for various investigations

Study design: This is a hospital based case control study.

Inclusion criteria: Documented cases of PCOS of reproductive age group (15-45 years) with their informed consent.

Exclusion Criteria:

- Age < 25 years or > 45 years.
- Pregnancy.
- Clinical or electrocardiographic evidence of coronary artery disease, a family history of coronary artery disease or history of smoking
- Other endrinopathies eg. Hypothyroidism, Cushing syndrome.
- Patients taking oral contraceptives, glucocorticoids, antiandrogens, ovulation induction agents, hypolipidemic drugs.

- PCOS patients having HOMA-IR ≥2.5.

Sample size:
Cases- 150 PCOS patients (15-45 years)
Controls- 150 normal age matched subjects.

Procedure:
Patients who readily participated in the study voluntarily, were selected on the basis of inclusion and exclusion criteria, after proper consent. About 10 ml of venous blood was collected from each of study and control group at 12 hour fasting in early morning with proper aseptic technique- 8 ml blood was taken in Plain vial with clot retractor and rest 2 ml will be taken in a Fluoride vial .The sample taken in clot activator without anticoagulant were allowed to clot and then all the tubes were centrifuged at 1500 rpm speed for 3-5 minutes for separation of serum and plasma. After separation serum was stored at -20° C in the freezer compartment and plasma was stored at 2-8°C until analysis .All the tests were done with serum obtained from clotted blood except fasting plasma glucose which was done with plasma.

1) Total Cholesterol, HDL Cholesterol, LDL Cholesterol and Triglyceride were measured by commercial kits in COBAS 6000.
2) hs CRP was measured by Immunoturbidimetry in COBAS 6000.
3) Insulin resistance was calculated from fasting serum insulin and fasting plasma glucose level with HOMA-IR formula (Homeostasis Model Assessment of Insulin Resistance).[10]

HOMA-IR was calculated using the following formula:

$$\frac{\text{Fasting glucose (mmol/l)} \times \text{fasting insulin (μIU/ml)}}{22.5}$$

Women were classified as being insulin resistant or not insulin resistant in accordance with defined cutoff points for HOMA-IR ≥2.5.

2) Serum LH, FSH, Insulin, Total testosterone levels were estimated by CLIA method.

STATISTICAL ANALYSIS
Data obtained were placed into a Microsoft excel sheet and then analyzed by IBM Statistics SPSS version 23, 2015. Student's unpaired t-test with Welch correction was applied to compare between the normally distributed numerical variables. Chi-square analysis was done to compare nominal variables. Correlation was calculated by Pearson correlation analysis.

In this study, p-value < 0.05 has been considered to be statistically significant.

RESULTS
No significant difference was found between the ages of cases and controls (p value 0.66) by independent t-test.

Table 1: Biochemical Markers Of Cardiovascular Risk

PARAMETERS	CASES (n=150) MEAN (SD)	CONTROLS (n=150) MEAN (SD)	p-value
TOTAL CHOLESTEROL (mg/dl)	156.25 (12.78)	145.49 (12.70)	<0.001
HDL CHOLESTEROL (mg/dl)	38.23 (7.81)	49.48 (5.41)	<0.001
LDL-CHOLESTEROL (mg/dl)	107.20 (10.15)	80.11 (11.20)	<0.001
TRIGLYCERIDE (mg/dl)	142.27 (27.71)	114.72 (11.97)	<0.001
HS-CRP (mg/L)	15.66 (3.71)	4.60 (2.46)	<0.001

Table 2 : Correlation Of Serum Hs-crp Levels With Fasting Serum Total Cholesterol, Hdl-cholesterol, Ldl-cholesterol And Triglyceride Levels Of Cases.

Statistical Approach	Serum T.Cholesterol	Serum HDL-C	Serum LDL-C	Serum Triglyceride
Pearson Correlation Coeficient	0.690	-0.806	0.689	0.693

In the present study, corroborating with the previous studies [11,12] , serum Total Cholesterol was found to be higher in comparison to the healthy control groups and the Pearson correlation coefficient was positive with serum hs-CRP levels. HDL-Cholesterol was found lower in comparison to the healthy control groups and the Pearson correlation coefficient was negative with serum hs-CRP levels. In the present study, the mean LDL-C value was significantly higher than the healthy control group. That change in LDL-C also has significant positive correlation with serum hs-CRP levels.

In our study serum Triglyceride was found higher in comparison to the healthy control groups. The pearson correlation coefficient was found positive with serum hs-CRP levels. 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[13]

DISCUSSION
PCOS is a syndrome associated with hyperinsulinemia and hyperandrogenism. Hyperinsulinemia produces a cluster of CVD risk factors including dyslipidemia, IGT, hypertriglyceridemia, sd LDLc and reduced HDLc, as observed by Purohit et al.[14]

Bickerton et al [15] found no differences in surrogate markers linked to enhanced cardiovascular risk factors like total and high density lipoprotein cholesterol, triglycerides, apolipoprotein B-100, apolipoprotein A1, lipoprotein (a)), and sialic acid, fibrinogen, homocysteine, and C reactive protein (CRP) between patients with PCOS and weight matched controls . In this study, we have documented higher levels of total cholesterol, LDL-C, triglycerides lower levels of HDL-C, among women with PCOS compared with matched control subjects. One of the most important observations is that the differences in risk factors between PCOS women and control subjects are persisting even in normoinsulinemic subjects. Elevated hs-CRP was associated with cardiovascular risk factors in normoinsulinemic PCOS without metabolic syndrome. These patients need more intensive screening or treatment for this disease.

Based on our results and literary data, we propose that all women, when diagnosed with PCOS, should have at least their lipid profile values determined even in the absence of Insulin resistance. In addition, all these patients should have regular metabolic follow-up as a group at potential risk for early development of CHD. Further investigations, preferably prospective studies, are needed to elucidate the exact effect of dyslipidemia and the incidence of cardiovascular events in PCOS in the next decades.

REFERENCES
1. Carmina E, Lobo RA. Polycystic ovary syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. J Clin Endocrinol Metab 1999;84:1897-9.
2. Wojciechowski P, Lipowska A, Rys P, et al. Impact of FTO genotypes on BMI and weight in polycystic ovary syndrome: a systematic review and meta-analysis. Diabetologia 2012;55:2636-45.
3. Azziz R, Carmina E, Dewailly D et al. Position statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society Guideline. J Clin Endocrinol Metab. 2006;91(11):4237–45.

4. Dahlgren E, Janson PO, Johansson S et al. Polycystic ovary syndrome and risk for myocardial infarction. Acta Obstet Gynecol Scand 1992;71:599–603.
5. Talbott EO, Guzick DS, Sutton-Tyrrell K et al. Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. Arterioscler Thromb Vasc Biol 2000;20:2414–21.
6. Blake GJ, Ridker PM. Inflammatory bio-markers and cardiovascular risk prediction. J Intern Med 2002;252:283–94.
7. C.C. Kelly, H. Lyall, J.R. Petrie et al. Low grade chronic inflammation in women with polycystic ovarian syndrome, Journal of Clinical Endocrinology and Metabolism 86 (2001), 2453–2455.
8. HF Escobar-Morreale, G. Villuendas, J.I. Botella-Carretero et al. Obesity, and not insulin resistance, is the major determinant of serum inflammatory cardiovascular risk markers in pre-menopausal women, Diabetologia 2003;46:625–633.
9. M. Mohlig, J. Spranger, M. Osterhoff M et al. The polycystic ovary syndrome per se is not associated with increased chronic inflammation, Eur J Endocrinol 2000;50:525–532.
10. Matthews DR, Hosker JP, Rudenski AS et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–419.
11. Austin MA, King MC, Vranizan KM et al. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. Circulation 1990; 82:495–506.
12. Rizzo M, Berneis K. Lipid triad or atherogenic lipoprotein phenotype: a role in cardiovascular prevention. J Atheroscler Thromb 2005;12:237–239.
13. Schannwell CM, Strauer BE. Hypertension and cardiac failure. Internist (Berl). 2007;48(9):909-20.
14. Praveen Sharma , Aditi Gupta , Purvi Purohit. Cardiovascular Risk Factors Insulin Resistance [IR], High Sensitivity C-reactive Protein (hs-CRP) and Fibrinogen in PreMenopausal women with Polycystic ovarian syndrome (PCOS). Journal of Cardiovascular Disease Research 2015; 6(2): 67-71.
15. AST Bickerton, N Clark, D Meeking et al. Cardiovascular risk in women with polycystic ovarian syndrome (PCOS). J Clin Pathol 2005;58:151–154.