C-Reactive Protein Level in Polycystic Ovary Syndrome – A Cross Sectional Study in Kerala, India

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ABSTRACT

BACKGROUND

Polycystic ovary syndrome (PCOS) is one of the main causes for infertility. Many studies show that PCOS is associated with production of some inflammatory factors like CRP and IL - 18, which has a prognostic significance in analysing the risk of future cardiovascular diseases (CVD). American heart association (AHA) has put forward C - reactive protein (CRP) as a clinically useful marker for risk of cardiovascular diseases. Previous studies show an increased incidence of high levels of CRP in PCOS patients as compared to controls. If such an association is proved in our population, CRP can be used as an ideal marker to screen apparently normal young PCOS women for CVD. So we wanted to assess the CRP levels in PCOS through this study.

METHODS

This was a cross sectional study conducted in the outpatient departments of Infertility and Gynaecology in a tertiary care hospital of South India, from 2018 to 2019. It was conducted among women of age 25 - 35 years who were diagnosed to have PCOS according to Rotterdam's criteria, satisfying inclusion criteria. Women of age 25 - 35 years without PCOS were taken as comparison group. The study was conducted in 50 PCOS women and 50 women without PCOS were taken as comparison group. Data was obtained with the help of appropriate questionnaire and laboratory investigations. CRP level was assessed by Immunoturbidimetric method.

RESULTS

CRP was found to be significantly elevated ($P < 0.01$) in PCOS (1.87 ± 2.16) when compared to women without PCOS (0.16 ± 0.4).

CONCLUSIONS

CRP levels were significantly higher in PCOS women as compared to women without PCOS. Thus CRP can be used as tool to assess inflammatory state in PCOS.

KEYWORDS

Polycystic Ovary Syndrome, C Reactive Protein, Inflammation

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BACKGROUND

In this modern world polycystic ovary syndrome is responsible for 80 % of the anovulatory infertility cases.[1] Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting approximately 6 - 8 % of the women of reproductive age group worldwide.[2] In a study conducted in Central India among 840 girls of age 18 to 21 years, the estimated prevalence was 8.34 %.[3]

PCOS is defined by the presence of hyperandrogenism (clinically and / or biochemically) and / or chronic anovulation in the absence of specific adrenal and / or pituitary disease.[4]The main endocrine abnormalities in PCOS include accelerated gonadotropin releasing hormone (GnRH) pulsatile activity causing hyper secretion of luteinizing hormone (LH) resulting in hyperandrogenism.[5]

Recent studies suggest PCOS as a state of chronic inflammation marked by increased levels of inflammatory markers like IL - 18, CRP, endothelin - 1, Plasminogen activator inhibitor-1 (PAI – 1) etc.[6] The exact cause for this inflammation is not known. But over the years this inflammatory state has a very detrimental effect on body especially in blood vessels leading to vascular occlusion. Some prospective studies have shown 75 % of PCOS women develop diabetes mellitus by 40 years.[7]

C reactive protein (CRP) is one of the main markers of inflammation. It is made up of 224 amino acids, and has a molecular mass of 25,106 Da. It assembles into stable pentameric structure with a discoid shape in the serum. CRP is an acute phase reactant produced by liver cells under stimulatory effect of pro - inflammatory cytokines like Interleukin - 6 and tumour necrosis factor.[6]

Elevated level of CRP is an independent marker of risk for developing cerebrovascular and cardiovascular morbidities.[8] CRP also has some direct effect on vascular wall contributing to formation of plaque, rupture and thrombosis. CRP contributes to an arterial pro - inflammatory and proatherosclerotic phenotype by directly up regulating adhesion molecules and chemokines in endothelial cells, vascular SMCs and monocytes. CRP also up regulates the expression of adhesion molecules which helps in atherosclerotic plaque formation in vessel wall.

Though many studies have been conducted worldwide, only limited studies have been conducted in Kerala to interrogate the inflammatory state in PCOS.

This study intends at determining whether C - reactive protein is elevated in PCOS women attending infertility OPD at a tertiary centre in south India. If such an association is proved in our setting, CRP level can be used along with routine gynaecological examination, as a cheap prognostic test to determine future risk of cardiovascular diseases. In such patients' early intervention strategies like control of inflammation, by diet, lifestyle modification or drugs can be initiated at the earliest.

Objectives

- To confirm the inflammatory state prevailing in PCOS patients by assessing the inflammatory marker - CRP in these patients.
- To assess CRP levels in young women with PCOS, at a tertiary care centre in south India and to compare these values with CRP levels of women without PCOS.

METHODS

Setting and Design

A cross sectional study was designed and conducted among PCOS patients attending Outpatient Departments of Infertility and gynaecology, in a tertiary care centre in south India from 2018 – 2019.

50 women of age 25 to 35 years newly diagnosed with PCOS (according to Rotterdam's criteria) were selected as cases. Women of same age group with normal menstrual cycles and no clinical evidence of hyperandrogenism, were taken as comparison group.

The study was conducted for a period of 1 year after obtaining clearance from institutional scientific committee and ethics committee, (IEC Number. 13 / 05 / 2017 / MCT). Written consent was taken from all participants and obtained information was kept confidential and used only for the purpose of the study.

Exclusion Criteria

Cases with history of hypertension, diabetes mellitus, coronary artery disease, endocrine abnormalities, recent history of fever, infection or inflammation were excluded. Consecutive cases of PCOS and women without PCOS satisfying inclusion criteria were selected till the required sample size was met. PCOS diagnosis was based on diagnostic criteria established by "The 2003 Rotterdam consensus workshop". PCOS was diagnosed when patients satisfied 2 out 3 criteria:

- Menstrual dysfunction.
- Presence of multiple ovarian cysts as diagnosed by ultra sonogram.
- Evidence of clinical / biochemical hyperandrogenism.

Hirsutism was evaluated by Ferriman – Gallwey score[9] (refer annexure 3). Nine areas of the body were assessed for hair growth. This includes:

1. Upper lip
2. Chin
3. Chest
4. Upper abdomen
5. Lower abdomen
6. Upper back
7. Lower back
8. Upper arms
9. Thighs

Hair growth was rated from 0 (no growth terminal hair - 4 (extensive terminal hair) in each of the nine locations. Total score range from 0 – 36.[9] A score greater than 8 was regarded as androgen excess. Other indicators of androgen excess such as acne and acanthosis nigricans were looked for.

For CRP estimation 2 ml of venous blood was collected and estimated using quantitative turbidimetric immunoassay method. Normal range kept at 10 mg / L.[10]

Sample size was calculated from previous study[11]
Boulman N, Levy Y, Leiba R, Shachar S, Linn R, Zinder O, et al. Increased C - Reactive Protein Levels in the Polycystic Ovary Syndrome : A Marker of Cardiovascular Disease. The Journal of Clinical Endocrinology & Metabolism. 2004; 89 (5): 2160 – 5

For assessment of CRP levels in PCOS, the formula used was

$$n = \frac{(z\ 1-\alpha/2)^2 \times s^2}{d^2}$$

Where (z 1 – α / 2) is the standard normal variate value taken as 1.96.
s is the standard deviation. (s = 7 based on the reference study)
d is the absolute precision. (d = 1.98)
The sample size obtained 47 (approximated to 50)
For comparison of CRP levels among women with PCOS and women without PCOS, the formula used is

$$N = 2 \times \frac{(z\ (1-\alpha/2)^2 + z\ (1-\beta)\ )^2 \times \sigma^2}{d^2}$$

where σ is the average of standard deviation of study group and comparison group. (4.72 based on reference study), d is the difference in means of these two groups.
The approximate sample size comes as 30. Since the sample size obtained using the first formula was more, sample size was taken as 50. So study was conducted in 50 PCOS women and 50 women without PCOS were taken as comparison group.

Statistical Analysis

The data obtained was expressed as mean ± standard deviation. Wilcoxon Signed Ranks test and paired sample test were done to test for the normality of data. Independent t test was carried out to compare quantitative parameters among different. P value < 0.05 was considered as threshold for statistical significance. Statistical analysis was performed by using statistical software package SPSS, version 20.0.

RESULTS

In this study, the mean age of the cases was 28.3 ± 3 years while that of comparison group was 28.4 ± 2.8 years. The mean CRP level among PCOS cases was found to be 1.87 mg / dl with a standard deviation of 2.16. The maximum value of CRP obtained was 8 mg / dl.

The mean CRP level among comparison group was 0.16 mg / dl with a standard deviation of 0.4. The maximum value of CRP among comparison group was 2 mg / dl. The difference was statistically significant with P value less than 0.01.

CRP Level	Case	Comparison Group
Mean	1.87	0.16
SD	2.16	0.40
Median	0.80	0.00
Minimum	0.00	0.00
Maximum	8.00	2.00

Table 1. Descriptive Statistics for CRP Levels among Cases and Comparison Group

Group	Mean	SD	N	t	P
Case	1.87	2.16	50	5.5	P < 0.01
Control	0.16	0.40	50		

Table 2. Comparison of CRP Levels between Cases and Comparison Group

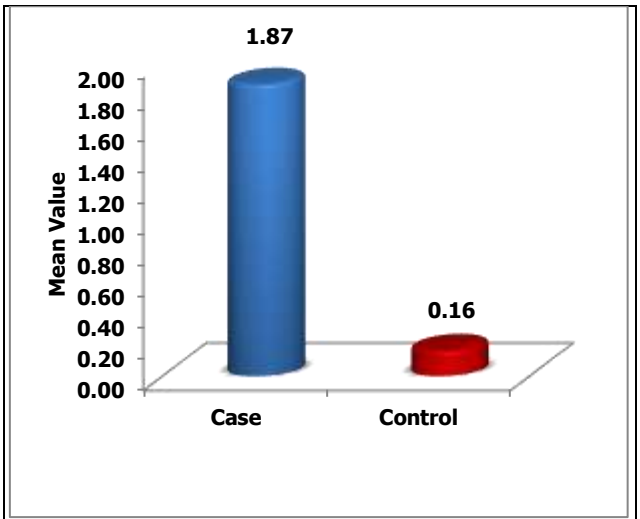

Figure 1. Comparison of CRP Levels among Cases & Comparison Group

DISCUSSION

Our study showed significant increase in CRP level among PCOS women with a P value less than 0.001. The mean CRP value among the 50 women diagnosed with PCOS according to Rotterdam criteria was found to be 1.87 ± 2.16 mg / dl. In this study the maximum CRP level obtained among the cases was 8 mg / dl. While in comparison group without PCOS the mean CRP level was 0.16 ± 0.4 g / dl.

This was in agreement with previous studies conducted by Boulman et al. in 116 PCOS patients.[11] In that study, the mean ± SD CRP concentration was 5.46 ± 7.0 mg / litre in the PCOS group and 2.04 ± 1.9 mg / litre in the controls. Mohamadin et al. conducted a similar study in 50 Saudi women with PCOS diagnosed using Rotterdam's criteria and found that a highly elevated hs-CRP level existed in PCOS patients.[12]

Prevalence of PCOS is highly variable ranging from 2.2 % to 26 % globally and is one of the leading causes of infertility. Studies done in South India and Maharashtra have reported prevalence (by Rotterdam's criteria) of 9.13 % and 22.5 % (10.7 % by Androgen Excess Society criteria).[13]

The pathophysiology of PCOS appears to be multifactorial and polygenic. Insulin resistance, hyperandrogenism and anovulation are the key features of PCOS. Insulin resistance is a central to the pathogenesis of PCOS, driving both hyperandrogenism and ovulatory dysfunction leading to clinical features.[14] Interaction of genetic, environmental, intra uterine and extra uterine

factors and alternative adaptations to energy excess plays a role in pathogenesis of insulin resistance. Abnormalities in insulin receptor signalling also contribute to insulin resistance. The resulting hyper insulinemia stimulate Cytochrome P450C17α activity in the ovary and adrenal causing increase steroidogenesis.[15]

Recently a state of chronic low grade inflammation has been recognized in PCOS. The clear cut aetiology of this inflammation is not understood. The main markers of inflammation associated with PCOS include raised total leucocyte count,[16] Tumour necrosis factor (TNF) – α, IL - 6, CRP etc. Inflammation has a very crucial role in pathogenesis of insulin resistance and hyperandrogenism. Also a chronic inflammatory state prevailing in PCOS can lead to development of vascular abnormalities causing cardiovascular diseases.[17] CRP has shown to alter the vessel wall characteristics and is observed to be directly involved in pathogenesis of atherosclerotic plaque formation.

Escobar morreale et al. in a meta analysis of the mean differences in CRP, IL - 6, and TNF - α indicates that CRP is a circulating marker of the pro inflammatory state in PCOS.[18] They also established a twofold elevation in circulating CRP in women with this disorder compared with controls.

The exact aetiology behind the development of inflammation in PCOS is unknown. Still some of the factors leading to inflammation have been suggested by some studies. One of the major causes is presence of inflammatory genotypes in PCOS patients such as those encoding TNF α, IL – 6 etc.[19] It was recently found that polymorphisms in the genes encoding TNF - α, type 2 TNF receptor, IL - 6, and the IL - 6 signalling molecule gp130 are associated with hyperandrogenism and PCOS,[20] or influence hyperandrogenic phenotypic traits.[21] Further, it has been noted that in PCOS patients there has been a significant reduction of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, indicating the presence of oxidative stress in these women.[22] There is a vicious cycle existing between inflammation and oxidative stress.

Chronic inflammation in PCOS is now recognized as one of main contributing factor to insulin resistance and hyperandrogenism.[23] Inflammatory mediators like TNF - α has the capacity to stimulate increased serine phosphorylation of insulin receptor that leads to insulin resistance.

Many researchers studying about inflammation in PCOS women had observed that low grade inflammation in PCOS is involved in the pathogenesis of T2DM and cardiovascular disease.[17,24,25] Ridker et al. conducted a prospective study to find the association between CRP, metabolic syndrome, and incident cardiovascular events among 14719 apparently healthy women for a 8 - year period for myocardial infarction, stroke, coronary revascularization, or cardiovascular death. It was suggested that measurement of CRP added clinically important prognostic information to the development of metabolic syndrome.[26] Majority of studies addressing the status of chronic low - grade inflammation in PCOS have focused on the measurement of circulating C - reactive protein (CRP).[27]

CRP levels also correlate with several other components of the metabolic syndrome such as fasting insulin, micro albuminuria, and impaired fibrinolytic activity. CRP also increases the release of endothelin - 1 and up regulates adhesion molecules and chemo attractant chemokines in endothelial cells and vascular SMC and has a role in development of atherosclerosis.[28]

From the results of this study it is clear that an inflammatory state prevails in PCOS subjects when compared with healthy counterparts of same age.

If potentially at risk candidates of PCOS are found at younger age itself, the inflammatory state can be lowered by taking adequate measures at the right time. Aerobic exercise has an important role in improving the metabolic profile and BMI and thus reducing the CRP levels. A 16-week exercise training program conducted among sedentary women demonstrated a significant decrease in CRP levels.[29] Antioxidants like vitamin E and D also has a role in reducing CRP levels.[28] Hypoglycaemic drugs like metformin and rosiglitazone have shown to reduce CRP levels. Prasad et al. has shown that statins like atorvastatin has the ability to reduce CRP levels in patients with impaired glucose tolerance.[28]

Thus if this state of inflammation is diagnosed earlier, future risk of cardiovascular morbidities can be prevented.

CONCLUSIONS

The present study showed a significant elevation in CRP level in patients with PCOS. It confirmed the inflammatory state prevailing in PCOS subjects when compared with their healthy counterparts.

Limitations

- The study was conducted from a limited population in a single tertiary care centre.
- Other factors which raise the CRP levels were assessed only by proforma and clinical examination. Chances of missing subclinical or hidden infections and malignancies may be there.
- Another limitation is time restraint. More prospective studies have to be done to follow up PCOS women with high CRP to look for vascular disorders in future.

Future Perspectives

- Constant evaluation of CRP levels in addition to gynaecological investigations in PCOS can be useful in finding out high risk cases and in giving adequate treatment.
- Also more interventional studies have to come to find out a definitive treatment protocol to the inflammatory state prevailing in PCOS, thus reducing the cardiovascular risk.
- Long term follow up studies should be initiated to study natural progression of disease and development of diabetes or cardiovascular disorders.

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