Role and Usefulness of C-reactive Protein in Women with Polycystic Ovarian Syndrome

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Authors’ contributions

This work was carried out in collaboration among all authors. Author IPA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SN and AKS managed the analyses of the study. Authors IPA and AM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a complex medical condition characterized by elevated androgen levels, menstrual irregularities, and small cysts on one or both ovaries. The prevalence of PCOS is 6 to 10% in women. C-reactive protein (CRP) is an acute-phase protein produced by the hepatic cells and it's levels increased in inflammation that increases interleukin-6 by macrophages and T cells.

Aims and Objective: A correlative study of role and usefulness of CRP in women with PCOS and its correlation with different biochemical parameters.

Materials and Methods: This was hospital based case-control study carried out among PCOS was conducted in the Department of Obstetrics & Gynaecology, Index Medical College Hospital, Indore. This study was conducted from 1st January 2018 to 31st December 2019. A total of 260 subjects with age group between 15 to 45 years were divided into two group; cases (130) and controls (130).
**INTRODUCTION**

Polycystic ovary syndrome (PCOS) is a complex medical condition that occurs in reproductive age group, characterized by elevated androgen levels, menstrual irregularities, and/or small cysts on one or both ovaries [1]. The polycystic ovary syndrome is a disorder that is characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphologic features. As defined by the diagnostic criteria of the National Institutes of Health (i.e., hyperandrogenism plus ovulatory dysfunction), "classic" polycystic ovary syndrome affects 6 to 10% of women of reproductive age, but the prevalence may be twice as high under the broader Rotterdam criteria [2]. The manifestations of androgen excess (e.g., hirsutism) may cause substantial distress in patients, and the polycystic ovary syndrome is the most common cause of anovulatory infertility. The manifestations of PCOS are not confined to the gynaecological sphere; women afflicted by this disease show an increased prevalence of several co-morbidities, including obesity, dyslipidemia, hypertension, metabolic syndrome (MS), and type 2 diabetes mellitus (DM2) in comparison with women without PCOS. These features, along with other alterations such as endothelial dysfunction and a chronic low-grade inflammatory state, underlie the greater risk of developing cardiovascular disease and increased all-cause mortality observed in these subjects [3].

C-reactive protein (CRP) is a ring-shaped, pentameric acute-phase protein found in plasma, and its level increased in response to inflammation. It’s produced by the hepatic cell, and it’s level increased following interleukin-6 secretion by macrophages and T cells. Its major role is to binding to lysophosphatidyl-choline expressed on the surface of dead cell or dying cells so as to activate the complement system via the complement component 1q (C1Q) complex. CRP may be a phylogenetically conserved protein, with homologs in vertebrates and lot of invertebrates that involve within the systemic response to inflammation. Its plasma concentration increases during inflammatory states, so that it has been use for the diagnosis purposes. CRP may be pattern recognition component, that binding to specific molecular configurations that are typically exposed during death cell or found on the cell surfaces of pathogens. Its level increase within hours after tissue injury or infection suggests that it contributes to host defence and that it is part of the innate immune response. A high level of CRP seen in the blood indicates that there may be an inflammatory process occurring in the body. Inflammation itself isn’t typically a problem, but it can indicate host of other health concerns, including infection, arthritis, kidney damage, and pancreatitis. High CRP levels indicate increased risk for coronary artery disease, which may cause a cardiac attack.

While the etiologic of PCOS and the casual association with inflammatory markers is not clear yet, some studies have investigated the

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**Results:** Among a total of 260 cases, based on clinical and different biochemical parameters, 130 were diagnosis with PCOS and 130 were apparently healthy women. The mean ±SD of various parameters among PCOS cases were; body mass index (BMI) 32.97±8.466, P (<0.001) total cholesterol (TC) 188.42±31.126, P (<0.001), triglyceride (TG) 134.43±50.01, P (<0.001), high density lipoprotein (HDL) 36.29±9.55 TC/HDL ratio, 5.54±1.865 serum CRP, 3.41±0.94 versus BMI 22.87±2.470, P (<0.001), TC155.42±26.333, TG 110.00±42.19, HDL 41.22±10.912, TC/HDL ratio 4.08±1.39, serum CRP 2.25±0.83 P (<0.001) in healthy control.

**Conclusion:** In this study, the role of inflammation and different biochemical markers were studied among PCOS. It was found that a majority of PCOS patients were obese, having insulin resistance. The levels of CRP as a marker of chronic low grade inflammation were higher in newly diagnosed PCOS as compared to the controls. The CRP values correlated well (statistically significant) with increased in BMI and age.

**Keywords:** Polycystic ovary syndrome; PCOS; CRP; BMI; total cholesterol; triglyceride.

**ABBREVIATIONS**

- **BMI** : Body mass index;
- **CRP** : C-reactive protein;
- **CVD** : Cardiovascular disease;
- **HDL** : High density lipoprotein;
- **HDL-C** : High density lipoprotein cholesterol;
- **CRP** : C-reactive protein;
- **LDL** : Low density lipoprotein;
- **LDL-C** : Low density lipoprotein cholesterol;
- **PCOS** : Polycystic ovary syndrome;
- **LDL** : Low density lipoprotein;
association between CRP with insulin resistance and PCOS. They have shown positive correlation between the increase in CRP with insulin resistance and PCOS while the association between PCOS was reported to be stronger [4],[5]. The present study aimed to evaluate the serum level changes of CRP in PCOS in comparison with healthy controls matched for age, BMI, blood pressure and marital state. In other words, we aimed to determine the changes in CRP in patients who do not have obesity. In addition we wanted to determine the association between CRP with PCOS.

2. MATERIALS AND METHODS

This was hospital based cross-sectional study carried out among Polycystic Ovarian Syndrome was conducted in the Department of Obstetrics & Gynecology, Index Medical College Hospital and Research Center, Indore from the 1st January 2018 to 31st December 2019. A total of 260 subjects with age between 15 to 45 years (reproductive age group) were divided into two groups as cases and control. About 130 patients was diagnosis of women with PCOS, and 130 apparently apparently healthy women were enrolled.

2.1 Inclusion Criteria

1. History of oligomenorrhea and/or anovulation,
2. Clinical (hirsutism, Ferriman-Gallweyscore≥8) and /or biochemical signs of hyperandrogenism serum testosterone ≥ 2.5nmol/L or DHEAS ≥ 8.5µmol/L [6].
3. Ultrasonographic evidence of polycystic ovaries and exclusion of other etiologies (congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome).

2.2 Exclusion Criteria

1. History of connective tissue diseases, immunological diseases, myocardial disease,
2. Malignancy, familial hyperlipidemia, chronic liver and kidney diseases women on chemotherapy.
3. On medicine like oral contraceptive pills and long term systemic corticosteroids. Women with history of hyperprolactinemia, type 2 diabetes mellitus, type 1 diabetes mellitus, late onset congenital adrenal hyperplasia, thyroid dysfunction, Cushing’s syndrome or patients taking medications that alter the hormonal or biochemical profiles.

2.3 Blood Samples

Approximately 5ml overnight fasting blood sample was collected from each participant's in plain vial for serum testing, EDTA (k3EDTA) vial for estimation of haematological and biochemical parameters citrated vial for CRP assessment.

2.4 Methods of Estimation

The estimation of various parameters were done as per following.

2.5 Estimation of CRP

It was done by using isotonic saline dilutions of the test specimen positive in the qualitative method, one drop of RHELAX-CRP latex reagent was added to the drop of test specimen, mixed it with stick and observing for agglutination macroscopically at two minutes.

2.6 Estimation of Blood Glucose

It was done by using standard glucose oxidase-peroxidase method (Erba EM 200).

2.7 Estimation of Lipid Profile

It was done by using the fasting blood sample based upon the Spectrophotometric principle and with the help of auto analyser machine (Erba EM 200).

2.8 Statistical Analysis

For statistical analysis a SPSS v20 (IBM©, Chicago, IL, USA) software was used. Quantitative results have been provided as a mean and standard deviation (SD) and have been measured, if appropriate, by the ANOVA (F) method. Different demographic and test parameters were calculated and tabulated in the master chart and analyzed. The data normality were also checked and nullified as per principle. The qualitative results is provided as numbers and percentages and contrasted, if possible, with the Chi-square (X2) method. The value of \( P \) was
considered statistically significant, when it came < 0.05.

3. RESULT

The present study has been conducted in Department of Biochemistry in collaboration with the OPD of Department of Obstetrics & Gynaecology, Index Medical College Hospital and Research Centre, Indore, MP. In the present study total 260 women with age group 20-45 years were enrolled as study subjects out of which, 130 apparently apparently healthy women were taken as control and 130 diagnosed of PCOS were taken as case. The test parameters were tabulated as per the master chart. The results were expressed in terms of mean±SD.

In the Table 1, the mean±SD of age was 25.19 ± 3.54 in cases, and 27.49 ±5.158 in controls and the maximum numbers of patients (46.1%) were in the age group; between 26–30 years.

When marital status was considered, out of the 130 cases, 119 were married and 11 patients were unmarried. Similar in marital status, out of 130 control, 107 were married and 23 were unmarried. The percentage of patients in the study who did married was (86.9%) and who did not married was (13.1%) as shown in Table 2.

The Mean ± SD of body mass index is 32.97±8.466 and 22.87±2.470 in case and control respectively and this is statistically significant (P <0.001) as shown as Table 3.

The comparisons between various parameters of lipid profile were correlated among PCOS cases as shown in Table 4. The mean±SD of cases and controls for total cholesterol was 188.42±31.126, and 155.42±26.333 respectively. The mean±SD of triglycerides of cases and controls was 134.43±50.01 and 110.00±42.19 respectively. The mean±SD of high density lipoprotein was 36.29±9.553 and 41.22±10.912 for cases and controls respectively. The mean±SD of total serum cholesterol/high density lipoprotein ratio for cases and control was 5.54±1.865 and 4.08±1.39 respectively as shown in Fig. 1.

<table>
<thead>
<tr>
<th>Table 1. Age distribution in the study subject with PCOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups</td>
</tr>
<tr>
<td>15-20</td>
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<tr>
<td>21-25</td>
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<tr>
<td>26-30</td>
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<tr>
<td>31-35</td>
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<tr>
<td>&gt;35</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Mean ±SD</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Marital status of the study subjects</th>
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</thead>
<tbody>
<tr>
<td>Marital status</td>
</tr>
<tr>
<td>Married</td>
</tr>
<tr>
<td>Unmarried</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3. BMI of the study subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>BMI</td>
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</tbody>
</table>

<table>
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<tr>
<th>Table 4. Lipid parameters of study subjects</th>
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</thead>
<tbody>
<tr>
<td>Lipid parameters</td>
</tr>
<tr>
<td>Total cholesterol mg/dl</td>
</tr>
<tr>
<td>Triglyceride mg/dl</td>
</tr>
<tr>
<td>High density lipoprotein mg/dl</td>
</tr>
<tr>
<td>Total cholesterol/HDL ratio</td>
</tr>
</tbody>
</table>
Hirsutism, an indicator of hyperandrogenism, was present in about 83.1% of patients as shown in Table 5. There was a significant difference (P<0.001) between cases and controls with regards to hirsutism.

The mean ±SD of serum CRP, an inflammatory marker, is compared in the Table 6 between cases and controls. The mean ±SD of serum CRP between cases and controls is 3.41±0.94 and 2.25±0.83 respectively, and that association between these two groups was statistically highly significant (p<0.001).

In the Table 7, the Pearson’s correlation between various clinical parameters with CRP is done. CRP shows positive correlation with FBS, triglyceride, HDL, BMI, systolic BP and diastolic BP. But in case of FBS and HDL it is positive correlative but it is statically insignificant with P value 0.983 and P value 0.983 respectively. In case of cholesterol HDL ratio and post-prandial blood sugar it is negatively correlative with R value -0.074 and R-value was -0.07 but it is statically significant (P <0.0235) and (P 0.262) with CRP respectively. When total cholesterol compared with CRP it is negatively correlative with R value -0.033 and it is statically insignificant with (P 0.591).

4. DISCUSSION

In our study we have enrolled a total of 260 cases, based on clinical and different biochemical parameters, 130 PCOS cases and 130 were healthy controls. PCOS is a prevalent reproductive and metabolic disorder with variable phenotypes and an underlying pathophysiology that is still not completely understood. While the earliest description of the polycystic ovary dates back to the 17th century [7]. Women with PCOS are predisposed to increased visceral adiposity and this appears to be across all categories of BMI. Using dual x-ray absorptiometry (DEXA) it has been shown that subjects with PCOS had similar percentage of total and trunk fat but higher percentage of central abdominal fat compared with weight-matched controls [8]. The presence of increased visceral adipose tissue is associated with insulin resistance, hyperglycaemia and dyslipidaemia which as mentioned above are co-morbidities associated with PCOS. The visceral adipocytes exert these effects in a paracrine and endocrine manner via the secretion of a number of molecules some of which are markers of inflammation [8]. Low grade chronic inflammation in women with PCOS occurs, especially associated with obesity. The manifestations of chronic inflammation as evidenced by increases of CRP, pro-inflammatory cytokines and chemokines, white blood count (WBC), oxidative stress and various markers of endothelial inflammation were important factors [9].

Our study shown maximum number of polycystic ovarian syndrome was seen in age group between 26-30 year which was 46.1% case and 35.5% control similar types of study was done by Ramanandet et al. [10]. This study find young PCOS patients were studied and find maximum number of women seen in polycystic
ovarian syndrome was seen in 23-26 age group.

Table 5. Status of hirsutism the study subjects

<table>
<thead>
<tr>
<th>Hirsutism</th>
<th>Cases</th>
<th>Controls</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>22(16.9%)</td>
<td>130(100.0%)</td>
<td>152(58.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Present</td>
<td>108(83.1%)</td>
<td>0(0.0%)</td>
<td>108(41.5%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>130 (100.0%)</td>
<td>130 (100.0%)</td>
<td>260 (100.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Serum CRP of the study subjects

<table>
<thead>
<tr>
<th>Serum CRP</th>
<th>Cases</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum CRP</td>
<td>3.41±0.94</td>
<td>2.25±0.83</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 7. Pearson correlation of serum CRP with clinical variables in cases

<table>
<thead>
<tr>
<th>Correlation between serum CRP with different clinical variables</th>
<th>R value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum CRP vs BMI &lt; 20kg/m2</td>
<td>0.264</td>
<td>0.262</td>
</tr>
<tr>
<td>Serum CRP vs BMI &gt;20kg/m2</td>
<td>0.047</td>
<td>0.473</td>
</tr>
<tr>
<td>Serum CRP vs Age &lt;25 years</td>
<td>0.045</td>
<td>0.634</td>
</tr>
<tr>
<td>Serum CRP vs Age &gt;25 years</td>
<td>0.130</td>
<td>0.118</td>
</tr>
<tr>
<td>Serum CRP vs FBS</td>
<td>0.001</td>
<td>0.983</td>
</tr>
<tr>
<td>Serum CRP vs PPBS</td>
<td>0.07</td>
<td>0.262</td>
</tr>
<tr>
<td>Serum CRP vs TC</td>
<td>0.033</td>
<td>0.591</td>
</tr>
<tr>
<td>Serum CRP vs TG</td>
<td>0.044</td>
<td>0.482</td>
</tr>
<tr>
<td>Serum CRP vs HDL</td>
<td>0.023</td>
<td>0.706</td>
</tr>
<tr>
<td>Serum CRP vs SC/HDL ratio</td>
<td>0.074</td>
<td>0.235</td>
</tr>
<tr>
<td>Serum CRP vs Systolic BP</td>
<td>0.081</td>
<td>0.194</td>
</tr>
<tr>
<td>Serum CRP vs Diastolic BP</td>
<td>0.138</td>
<td>0.026</td>
</tr>
</tbody>
</table>

This study also explored that out of the 130 cases, 119 married and 11 unmarried women was participated and in control group of participant out of 130, 107 were married and 23 were unmarried and the overall percentage of patients in the study who did marriage was 86.9% and who did not marriage was 13.1%, which was statistically significant (P<0.027) these values were similar in a study conducted by Navid et al. That suggested according to the effect of PCOS and adjustment of all demographic variables, marital satisfaction in housewives was lower than employed women. Additionally, marital satisfaction in infertile women with non-academic educational levels was higher than in women with academic educational levels [11].

Our study shown that body mass index was significant correlated with polycystic ovarian syndrome (p<0.001) and the mean±SD was 32.97±8.466 and 22.87±2.470 in case and control group respectively our study also show mean BMI was higher in case in compare to control which was similar to the study conduct by Sharma et al. This study reported women with PCOS had a significantly higher BMI, higher mean of systolic BP, diastolic BP compare to the normal control women [12].

In this study we did shows comparisons between various parameters of lipid profile in PCOS patient was statistically significant (P<0.001) serum levels of total cholesterol, triglyceride, high density lipoprotein and total cholesterol/ high density lipoprotein ratio between cases and controls group was compare. The mean±SD of lipid profile of cases participant was TC-188.42±31.126, TG-134.43±50.01, HDL-36.29±9.55 and HDL ratio, was 5.54±1.865, and in control participants it was 155.42±26.333, 110.00±42.19, 41.22±10.912, 4.08±1.39 respectively. These findings showed a significant higher level of different lipid parameters in PCOS patients as compared to apparently apparently healthy women. This study also highlighted that the, TC levels were above the threshold cut-off value and finding was similar to the observations made by Kiranmayee et al. The maximum numbers of women with PCOS demonstrated abnormal anthropometric parameters, and in more than 70% women, such as low levels of high-density lipoprotein (HDL) cholesterol and high levels of triglycerides and low-density lipoprotein cholesterol were seen. A significant
positive correlations seen between triglycerides, high-density lipoprotein, and cholesterol were seen [13]. Another author Halasawadekar et al. also reported that, TG and TC/HDL ratio were significantly high in PCOS group compared to the healthy control [14].

Hirsutism, an indicator of hyperandrogenism, in the present study seen among 108 (41.5%) of participants was seen in hirsutism out of this 108 (83.1%) was seen in case and no hirsutism was seen in control groups, similarly hirsutism was not seen among 22 (16.9%) women with PCOS. These finding showed that, hirsutism was seen in majority of PCOS patients and association was statistically significant. This study was similar to another study conduct by Khan et al., suggested that hirsutism was found in 70% of women with PCOS [15]. Another author, Yau et al. also found approximately 80% to 85% of women with hyperandrogenism clinically have hirsutism among PCOS [16].

The elevated level of C-reactive protein (CRP) was associated with hyper insulinemia, and it was a significant risk factor for cardiovascular diseases, and that plays a key role in the development of the PCOS. In this study finds mean±SD of CRP in case and control was 3.41±0.94 and 2.25±0.83 respectively. The level of CRP was significantly increased in PCOS cases as compared to controls, and difference was statistically highly significant, P value (<0.001). These high finding of CRP in the PCOS women suggest that women with PCOS may be indicate a risk for early onset of cardiovascular disease. These findings were similar to the study conduct by the author Kelly et al. and Boulman et al., which showed a significantly higher CRP levels in PCOS patients as compared to control group. The CRP concentrations, in this study, were significantly higher in PCOS patients as well as in the obese PCOS women compared to normal apparently apparently healthy women [17],[18].

Our study showed that, the mean CRP in the PCOS group was 3.41 (0.94) mg/dl, and in control group was 2.25 (0.83) mg/dl that was significant between two groups (p<0.001). The CRP levels among patients of PCOS were positively correlated with BMI (BMI<20kg/m2 (R=0.264) and BMI>20kg/m2 (R=0.047) FBS (R=0.001), HDL cholesterol (R=0.023) systolic blood pressure (R=0.081) and systolic blood pressure (R=0.138) and TG (R=0.044) and negatively correlated with the insulin sensitivity index that is cholesterol (R=-0.033), serum cholesterol/HDL ratio (R = -0.074) and PPBS (R= -0.07). But in case of FBS and HDL it was positively correlated, but it was statically insignificant with P value 0.983 and 0.983, respectively. In case of cholesterol/HDL ratio and PPBS it is negatively correlative with R value - 0.074 and -0.07 but it was statically significant with P value (<0.0235) and P (<0.262) with CRP respectively. When total cholesterol compared with CRP it is negatively correlative with R value -0.033, and it was statically insignificant with p (=0.591). Our study has similar findings with other different studies conducted by Boulman et al. and Al- Hakeim [18],[19].

This study find that 36.8% of the PCOS patients had CRP levels above 5 mg/liter, only 9.6% of the controls exhibited such CRP levels (P <0.001).Whereas, 46.5% of the PCOS patients had CRP levels above 3 mg/liter, only 27.7% of the BMI-matched controls exhibited such CRP levels (P = 0.005). A decreasing linear trend was found between CRP levels and control group as seen in previous studies [17],[18]. In another study conducted by Al-Youzbak et al. also reported similar finding in their study. This study revealed a significant high serum TC, LDL-C and TG, and lower serum HDL-C in PCOS women, when compared with healthy women and a significant high correlation was seen in the serum TG levels between case and control groups. There was a highly significant positive correlation between BMI and CRP in patients with PCOS in a study conducted among PCOS with metformin therapy [20].

In short, this study find that PCOS was associated with increased TC,TG,LDL-C and CRP concentrations, which supports the evidence that PCOS was associated with low-grade inflammation. The main predictor factors of increased CRP were; BMI and insulin resistance, but there was a relationship between lipid profiles in PCOS, so that inflammation may be mediated through adiposity concentration which was similar to the study conduct by Rudnicka et al. However, many other factors that can affect CRP and lipid profile such as diet, smoking, exercise, life style, blood pressure, and possibly statins levels, further studies are needed to understand the precise mechanism of chronic low-grade inflammation in women with PCOS [21].
5. CONCLUSION

In this present study, the CRP levels in the blood were compared in newly diagnosed cases of PCOS and controls. It was found that a majority of the PCOS patients were obese with insulin resistance. The levels of CRP as a marker of chronic low grade inflammation were higher in newly diagnosed PCOS cases as compared to the controls. The CRP values correlated well with increased in BMI and age. This could probably imply a state of insulin resistance, low grade inflammation, abdominal obesity and activation of the complement cascade in a majority of women with PCOS. Abdominal obesity, anovulation, hirsutism and irregular menstrual a common finding in women with polycystic ovarian syndrome can lead to increase in the release of the inflammatory mediators which can activate the complement cascade and thus show increased in the levels of CRP in women with PCOS. Thus in this study, the state of low grade inflammation seen in cases of PCOS, could strongly influence their risk for adverse polycystic ovarian syndrome and metabolic complications.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL CONSIDERATION

All the study process was started only after obtaining ethical approval (Ref-26/IEC/IMC/2020) from the institutional ethical committee. All the information about the participants was kept confidential. As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES