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Comparative Study of High Sensitive C Reactive Protein (hsCRP) and Complete Hematogram Levels Among Early and Prolonged PCOS Patients

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Abstract: Polycystic ovarian syndrome (PCOS) is a complex endocrine disorder which affects millions of women worldwide. It is a heterogeneous condition with various pathophysiological and many clinical presentations. To analyse the long term complications of PCOS a questionnaire was circulated to women volunteers with the age group ranged between 20-42 years. From which 30 early PCOS patients aged between (20-22) years and 30 prolonged PCOS patients aged between (40-42) years were selected based on Rotterdam criteria (2023) and the blood samples were analyzed for hsCRP and complete hematogram parameters. hsCRP (p<0.001), Leucocytes (p<0.01), and Neutrophils (p<0.001) showed significantly higher levels and RBC, Hemoglobin, MCHC, and MCH showed significantly lower levels (p<0.01) in prolonged PCOS patients when compared to the early PCOS patients. But the levels of Monocytes, Eosinophils, Basophils, Immature Granulocytes, Platelet Disc Width, Mean Platelet Volume, Platelet Volume, Platelet to Large Cell Ratio, Platelet Crit were found to be non significant.

Keywords: PCOS, hsCRP, Neutrophils, Endocrine disorder, Hematogram

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Introduction

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder which affects nearly 15% of reproductive women worldwide. It is characterized by infertility, irregular menstrual cycle, hirsutism and enlarged ovaries with multiple cysts. It is also considered as a genetic disorder because it is highly heritable (Dapas et al., 2020). The pathogenesis of PCOS is not clearly understood, although it is widely believed to be a multifactorial disorder because it include both genetic and environmental components. Three fourth of the women with PCOS suffers from infertility, obesity, insulin resistance and associated complications. This condition is common in the women of any ethnic but some study report that it is very popular condition.
mainly in south Asians and Hispanics. Rotterdam criteria is the most commonly used criteria for the diagnosis of PCOS from the year 2003. The criteria for PCOS includes the following three symptoms—(i) Anovulation or oligo ovulation; (ii) Hyperandrogenism; and (iii) Polycystic ovaries. When a women experience any two of these condition they said to have PCOS (Dewailly et al., 2020). The updated Rotterdam criteria includes that presence of more than 12 follicles in each ovary measuring 2–9 mm in diameter or an increased ovarian volume (>10 ml) (Dumesic et al., 2015). On long run PCOS may lead to several complications due to hormonal imbalances, among them the notable inflammatory marker includes hsCRP, a member of the pentraxin group that is composed of monomers comprising 221 amino acids, which is considered as the most powerful acute phase protein and the CRP gene is found in chromosome 1. The inflammatory cascade is started by infections or tissue injury that triggers macrophage activation and TNF-alpha and interleukin-1 production. Interleukin-6, a crucial CRP protein activator, is produced by fibroblasts and endothelial cells in response to TNF-alpha and interleukin-1. Adipocytokines and free fatty acids trigger low-grade inflammation, which plays a major role in the pathogenesis of metabolic syndrome, which leads to atherosclerosis and cardiovascular disease. So, measuring CRP levels is also a useful way to estimate the amount of chronic inflammation that raises the risk of cardiovascular disease. Moreover, studies have linked elevated hsCRP levels to type 2 diabetes, insulin resistance, and arterial disease. Women are also linked to high hsCRP levels when they have carotid artery stenosis. Increased level of Neutrophils and Leucocytes are seen in PCOS patients because Leucocytes have the ability to stick to the endothelium and go towards the bacterial center, where phagocytosis and the generation of ROS destroy the infection. Endothelial dysfunction is linked to an increase in leucocyte recruitment in some insulin resistance conditions, such as type 2 diabetes. In this regard, vascular stiffness can be more prominent in PCOS patients regardless of their age, blood pressure, or body mass index (BMI). Because PCOS patients often have high glucose levels, endothelial dysfunction is typically linked to the disease, which in turn is linked to Leucocyte-endothelium interactions and endothelial impairment. Two of the most prevalent subtypes of leukocytes are Neutrophils and Lymphocytes. Neutrophils have been regarded as the main participant in the systemic inflammatory response for much too long. On the other hand, immunoregulatory Lymphocytes are repressed in inflammatory and stress responses and an elevated Neutrophil count frequently indicates significant systemic inflammation. (Li et al., 2022).

The aim of the present study was to analyze the long-term complications of PCOS with regard to their inflammatory marker hsCRP and complete hematogram parameters of prolonged PCOS patients in comparison with early PCOS patients with similar BMI. Therefore the main objective of this study was to (i) calculate BMI, (ii) estimate hsCRP, (iii) estimate the complete hemotagram parameters such as Neutrophils, Total Leucocyte, RBC, %RBC, Red Cell Distribution Width, Monocytes, Eosinophils, Basophils, Immature Granulocytes, Hemoglobin, Mean Corpuscular Hemoglobin, Mean corpuscular Hemoglobin concentration, Mean Platelet Volume, Platelet volume, Platelet to large cell volume and Platelet Crit.

Materials and Methods

Study design and Sample collection:

A questionnaire was circulated to women volunteers with the age group between 20-42 years with written consent (ETHICAL CLEARANCE - ARCIIC/others/ 001/2001) and the PCOS patients were selected on Rotterdam criteria (2023). Based on the questionnaire, the BMI of the volunteers was calculated using the formula: BMI = weight (in kg)/height^2 (in meter). Patients with similar BMI were selected for the analysis. They were grouped into early and
prolonged PCOS patients based on the duration of PCOS. 5 ml of venous blood were collected by venipuncture method during their follicular phase and the following parameters were carried out:

**Estimation of hsCRP:**
hsCRP was assayed by Latex immunoturbidimetric assay in the fully automated analyzer (Beckman coulter).

**Complete hematogram parameters:**
(i) Estimation of RBC parameters such as Total Leucocytes, Neutrophils, RBC, Platelets, WBC, Eosinophils, Basophils, Monocytes by Hemocytometer.

(ii) Estimation of Hemoglobin was done by Drabkin's method.

**Statistical analysis:**
The values were expressed as Mean ± SD and the level of significance were arrived by Student t test using Microsoft Excel.

**Results and Discussion**

**Calculation of Body Mass Index (BMI):**
Table 1 shows the BMI of early and prolonged PCOS patients. The BMI of early PCOS patients was found to be 33.9 ± 1.0 and in prolonged PCOS patients the level of BMI was found to be 33.24 ± 0.9 and these levels were found to be not significant (p<0.5). Patients with similar BMI were selected for this study to exclude the complications associated with their body weight.

**Table 1: BMI of early PCOS patients Vs prolonged PCOS patients**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>PARAMETERS</th>
<th>EARLY PCOS PATIENTS</th>
<th>PROLONGED PCOS PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BMI</td>
<td>(33.9 ± 1.0)ns</td>
<td>(33.24 ± 0.9)ns</td>
</tr>
</tbody>
</table>

n=30; ns - not significant

**Determination of hsCRP:**
Table 2 shows the hsCRP level of early and prolonged PCOS patients. The hsCRP level of early PCOS patients was found to be 0.59 ± 0.14 and in prolonged PCOS patients hsCRP level was found to be 5.74 ± 0.8 and the levels are found to be highly significant (p<0.001)

Our results are supported by Zhang et al. (2016) who reported that significantly high correlation exist between hsCRP level and PCOS due to hyperandrogenism, hyperinsulinemia and insulin resistance seen in PCOS women. Osibogun et al. (2020) found that increased level of hsCRP level in PCOS women may increase the risk of cardiac disease.

Increased level of hsCRP is linked to chronic inflammatory conditions such as insulin resistance, type II diabetes mellitus and cardiovascular disorders. It is also linked with the inflammation within the arterial walls causing the deposition of fats and cause atherosclerotic plaques in prolonged PCOS women. Hence, consuming more healthy high fibre diet and increasing physical activities would reduce the degree of general inflammation in the body.

**Evaluation of RBC parameters:**
Table 3 shows the level of mean corpuscular Hemoglobin concentration, mean corpuscular Hemoglobin , % RBC, Hemoglobin, RBC, and Red cell distribution width.

The level of MCHC, MCH and %RBC in early PCOS patients was found to be 33.4±0.95, 31± 1.1,
Table 3: Hemoglobin and RBC parameters of Early PCOS patients vs prolonged PCOS patients

<table>
<thead>
<tr>
<th>S. No.</th>
<th>PARAMETERS</th>
<th>EARLY PCOS PATIENTS</th>
<th>PROLONGED PCOS PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mean corpuscular Haemoglobin concentration (MCHC) (g/l)</td>
<td>33.4 ± 0.95**</td>
<td>29.47 ± 1**</td>
</tr>
<tr>
<td>2</td>
<td>Mean corpuscular Haemoglobin (MCH) (pg)</td>
<td>31 ± 1.1**</td>
<td>27.05 ± 1.3**</td>
</tr>
<tr>
<td>3</td>
<td>% RBC</td>
<td>39.86 ± 1.1**</td>
<td>34.92 ± 1.1**</td>
</tr>
<tr>
<td>4</td>
<td>Hemoglobin (g/dl)</td>
<td>12.77 ± 0.6*</td>
<td>11.35 ± 0.7*</td>
</tr>
<tr>
<td>5</td>
<td>RBC (million/mm³)</td>
<td>4.24 ± 0.1*</td>
<td>3.51 ± 0.2*</td>
</tr>
<tr>
<td>6</td>
<td>Red cell distribution width (fl)</td>
<td>(43.51 ± 0.8)ns</td>
<td>(43.04 ± 0.7)ns</td>
</tr>
</tbody>
</table>

n=30; ns- not significant; * indicates p<0.01 - highly significant; ** indicates p<0.001 - highly significant

and 39.86 ± 1.1, respectively and in prolonged PCOS patients the results was found to be 29.47±1, 27.05± 1.3, and 34.92±1.1, respectively. The levels were highly significant (p<0.001).

The level of Hemoglobin and RBC in early PCOS patients was found to be 12.77 ± 0.6 and 4.24 ± 0.1, respectively and in prolonged PCOS patients the results were found to be 11.35 ± 0.7 and (3.51 ± 0.2, respectively. The levels were significant (p<0.01).

The level of Red cell distribution width in early PCOS patients was found to be 43.51±0.8 and in prolonged PCOS patients the level was 43.04±0.7 and they found to be not significant (p<0.5).

Bitzer et al. (2014) and Sabuncu et al. (2017) signifies the prevalence of anemia in adolescent PCOS patients and also hypothesized that the increased prevalence of hyperinsulinemia and insulin resistance in PCOS patients may be connected to the increase in the parameters of MCH and MCHC. Sathyapalan et al. (2018) and Cinar et al. (2022) suggested that increased level of MCH and MCHC is directly related with PCOS by emphasizing the rise in MCH and MCHC levels which may be caused by persistent low-grade inflammation, which is frequently seen in PCOS.

Due to hormonal changes, the prolonged PCOS patients undergo heavy and prolonged menstrual flow which might lead to insufficient amount of RBC and hemoglobin levels. Polyps can cause bleeding between periods as well as heavier flow during periods, heavy bleeding associated with disturbances in menstrual cycle and can increase iron deficiency, or the more severe iron deficiency anemia. To reduce the complications of blood loss and anaemia, vegetables such as spinach, beetroot, tomatoes and other leafy vegetables, nuts, raisins, whole grains and fruits such as pomegranate, apple and dates can be added in their diet.

Evaluation of WBC parameters:

Table 4 shows the level of Total Leucocytes, Neutrophils, Monocytes, Eosinophils, Basophils and Immature Granulocytes.

The level of Total Leucocytes in early and prolonged PCOS patients was found to be 3.95 ± 0.2 and 6.12 ± 1.1, respectively. The levels were found to be significant (p<0.01). The level of Neutrophils in early and prolonged PCOS patients was found to be 6.58±0.2 and 7.39±0.2, respectively. The levels were found to be highly significant (p<0.001). The level of Monocytes, Basophils, Eosinophils and Immature Granulocytes in the early PCOS patients was found to be 2.74 ± 0.5, 2.7 ± 0.2, 0.18 ± 0.008 and 0.28 ± 0.1, respectively and in prolonged PCOS
Table 4: White blood cell parameters of Early PCOS patients Vs prolonged PCOS patients

<table>
<thead>
<tr>
<th>S. No.</th>
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<th>EARLY PCOS PATIENTS</th>
<th>PROLONGED PCOS PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total Leucocytes /L</td>
<td>3.95 ± 0.2*</td>
<td>6.12 ± 1.1*</td>
</tr>
<tr>
<td>2</td>
<td>Neutrophils /L</td>
<td>6.58 ± 0.2**</td>
<td>7.39 ± 0.2**</td>
</tr>
<tr>
<td>3</td>
<td>Monocytes (%)</td>
<td>2.74 ± 0.5ns</td>
<td>3.05 ± 0.7ns</td>
</tr>
<tr>
<td>4.</td>
<td>Eosinophils (%)</td>
<td>2.7 ± 0.2ns</td>
<td>2.8 ± 0.3ns</td>
</tr>
<tr>
<td>5</td>
<td>Basophils (%)</td>
<td>0.18 ± 0.008ns</td>
<td>0.17 ± 0.07ns</td>
</tr>
<tr>
<td>6</td>
<td>Immature Granulocytes (%)</td>
<td>0.28 ± 0.1ns</td>
<td>0.32 ± 0.07ns</td>
</tr>
</tbody>
</table>

n=30; ns -non significant; * indicates p<0.01 -highly significant; ** indicates p<0.001 - highly significant

Table 5: Total and differential count of WBC Early PCOS patients and prolonged PCOS patients

<table>
<thead>
<tr>
<th>S. No.</th>
<th>PARAMETERS</th>
<th>EARLY PCOS PATIENTS</th>
<th>PROLONGED PCOS PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Platelet Disc Width</td>
<td>9.95±0.4ns</td>
<td>10.05±0.3ns</td>
</tr>
<tr>
<td>2</td>
<td>Mean Platelet Volume</td>
<td>9.85±0.2ns</td>
<td>9.75±0.2ns</td>
</tr>
<tr>
<td>3</td>
<td>Platelet volume</td>
<td>397.14±11.1ns</td>
<td>402.85±22.1ns</td>
</tr>
<tr>
<td>4</td>
<td>Platelet to large cell ratio (PLCR)</td>
<td>24.4±2ns</td>
<td>24.14±2.1ns</td>
</tr>
<tr>
<td>5</td>
<td>Platelet crit</td>
<td>0.41±0.03ns</td>
<td>0.42±0.01ns</td>
</tr>
</tbody>
</table>

n=30; ns -non significant

patients the levels was found to be 3.05±0.7, 2.8±0.3, 0.17±0.07, and 0.32±0.07, respectively. The levels were found to be not significant (p<0.5).

Cai et al. (2017) and Olga Papalou et al. (2016) estimated Neutrophil to Lymphocyte ratio as a potential biomarker and significantly higher level seen in PCOS patients. Shiet et al. (2013), Tang et al. (2019) and Khashchenko et al. (2022), supported our results by showing no significant increase in Monocytes, Eosinophils, Basophils and Immature Granulocytes levels.

From this study we could interpret that prolonged hormonal imbalance might have enhance their oxidative stress which could have elevated the levels of immune cells such as Leucocytes and Neutrophils. Increased WBC and Neutrophils levels are associated with their body weight and insulin resistance. Getting active and reducing their weights, eating more citrus fruits could alleviate the effects.

Estimation of Total and Differential WBC count:

Table 5 shows the level of Platelet Disc Width, Mean Platelet Volume, Platelet Volume, Platelet to Large Cell Ratio, Platelet Crit.

The level of Platelet Disc Width, Mean Platelet Volume, platelet volume, platelet to large cell ratio and Platelet Crit in early PCOS patients were found to be 9.95 ± 0.4, 9.85±0.2, 397.14±11.1, 24.4±2, and 0.41±0.03, respectively and in prolonged PCOS patients was found to be 10.05 ± 0.3, 9.75 ± 0.2, 402.85±22.1, 24.14 ± 2.1, and 0.42 ± 0.01. The levels were found to be not significant (p<0.5). Our results are supported by Guler et al. (2021) who have reported that no significant changes in Platelet Disc Width, Mean Platelet Volume, Platelet volume, Platelet to Large Cell Ratio and Platelet Crit.

Conclusion

As the prevalence of PCOS increasing tremendously and the associated complications were quite alarming, this study focused on the long term complications associated with it. A
questionnaire was circulated to identify the PCOS patients (Rotterdam criteria, 2023). Patients with similar BMI were selected for the analysis of hsCRP and complete hematogram parameters. It has been observed that the levels of hsCRP (p<0.001), Leucocytes (p<0.01), Neutrophils (p<0.001) showed significantly higher levels and RBC, Hemoglobin, MCHC, MCH showed significantly lower levels (p<0.01) in prolonged PCOS patients when compared to the early PCOS patients. But the levels of Monocytes, Eosinophils, Basophils, Immature Granulocytes, Platelet Disc Width, Mean Platelet Volume, Platelet Volume, Platelet to Large Cell Ratio, Platelet Crit were found to be non significant. As the results indicates that long term hormonal imbalance has an effect on important inflammatory marker and blood parameters in comparison with the early PCOS patients it is necessary to practice anti-inflammatory diet which includes more unsaturated fat viz (omega 3 and omega 6 containing foods) low calorie, low saturated fats and low glycemic index foods with lots of fibre intake. Hence, foods such as green leafy vegetables, fish, spices, virgin oil, whole grains, nuts and herbs are recommended along with mild to moderate exercise and enough sleep. Hence, early diagnosis, lifestyle modifications and medical consultations are recommended to avoid serious complications.

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**References**


Guler A and Demir I. (2021) Decreased levels of spexin are associated with hormonal and metabolic disturbance in subjects with polycystic ovary syndrome. J Obstetrics Gynaecol. 41(3): 408-413.


