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Vitamin E and CRP as biomarkers of inflammation in PCOS: A novel perspective

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Abstract
Polycystic Ovary Syndrome (PCOS) is one of the prevalent & widespread hormonal disorder affecting women of reproductive age, marked by hormone imbalances, irregular ovulation, and ovarian cysts. Research strongly suggests that persistent, low-grade inflammation plays a crucial role in the development and worsening of PCOS. This chronic inflammation is linked to problems like insulin resistance, higher levels of male hormones (hyperandrogenism), and an increased risk of heart problems.
C-reactive protein (CRP) is a trusted measure of general inflammation and is commonly elevated in women with Polycystic Ovary Syndrome (PCOS). This inflammatory state is often worsened by oxidative stress, which is an imbalance where damaging reactive oxygen species overwhelm the body's natural antioxidant defenses. Vitamin E, an essential fat-soluble antioxidant, serves as a measure of the body's overall antioxidant capacity and is known for its strong anti-inflammatory effects, helping to counteract this imbalance.
This study aimed to investigate the inflammatory status of newly diagnosed women with PCOS, measure their baseline Vitamin E levels in relation to CRP before they started any treatment. This case-control study, included comparing 88 women diagnosed with PCOS to 88 healthy women of similar age, their bood samples were analysed to estimate their serum CRP and Vitamin E concentrations.
The results showed that women with PCOS had significantly higher CRP levels (indicating more inflammation) and markedly lower Vitamin E levels (suggesting weaker protection) compared to the healthy group (both findings were statistically significant with $p<0.001$). This supports the idea that PCOS involves a greater inflammatory burden compromising balance between inflammatory and anti-inflammatory status. The clear inverse relationship between CRP and Vitamin E suggests that this imbalance might be contributing to the inflammation seen in PCOS. To conclude, the study highlights that women with untreated PCOS have high CRP and lower plasma Vitamin E levels, thus contributing to severity and progression of this condition. These findings emphasize the importance of considering them while managing PCOS.

Keywords: C-Relative Protein, PCOS, vitamin e, anti-inflammation

Introduction
Polycystic Ovary Syndrome (PCOS) is a complex and highly prevalent endocrine disorder affecting women of reproductive age worldwide. It is characterized by a triad of clinical features: menstrual irregularities, hyperandrogenism (elevated levels of male hormones), and the presence of polycystic ovaries on ultrasound [1, 2]. While these symptoms have traditionally been the focus of diagnosis and treatment, a growing body of evidence suggests that chronic, low-grade inflammation plays a pivotal and central role in the pathogenesis and progression of this condition [3, 4]. This inflammatory state is now understood to be more than just an associated feature; it is a fundamental driver that contributes to the hormonal and metabolic disturbances characteristic of PCOS [5].
The traditional view of PCOS has often centered on a vicious cycle involving insulin resistance and hyperandrogenism. However, recent research has illuminated the intricate relationship between these factors and systemic inflammation [6]. Chronic inflammation exacerbates insulin resistance by disrupting insulin signaling pathways, which in turn leads to hyperinsulinemia. The high levels of insulin then stimulate the ovaries to produce more androgens, further worsening the clinical picture of hyperandrogenism [7, 8]. This inflammatory state is not limited to the reproductive system, it is a systemic condition that contributes to the increased risk of long-term health complications such as type 2 diabetes, cardiovascular disease, and metabolic

syndrome, which are all more prevalent in women with PCOS [9, 10].

To objectively assess this inflammatory state, studies often rely on a variety of biomarkers. One of the most widely used and reliable indicators of systemic inflammation is C-Reactive Protein (CRP) [11, 12]. CRP is an acute-phase protein produced by the liver in response to pro-inflammatory cytokines like interleukin-6. Even in the absence of a clear infection or injury, elevated levels of CRP are indicative of a persistent, low-grade inflammatory burden [13]. Numerous studies have consistently demonstrated that women with PCOS have significantly higher basal levels of CRP compared to healthy women, thereby providing compelling evidence for a state of chronic inflammation in this population [14, 15]. The measurement of CRP is not only a research tool but also has clinical relevance, as it can serve as a valuable biomarker for assessing disease activity and risk stratification in women with PCOS [16].

Beyond the inflammatory state itself, another critical component in the pathophysiology of PCOS is oxidative stress [17]. Oxidative stress is an imbalance between the production of harmful reactive oxygen species (ROS) and the body's ability to neutralize them with antioxidants [18]. This imbalance leads to cellular damage and further intensifies the inflammatory cascade, creating a self-perpetuating cycle of damage and inflammation [19]. Therefore, the body's antioxidant capacity is a crucial factor in mitigating this harmful process. A compromised antioxidant defense system can leave the body vulnerable to the damaging effects of oxidative stress and the associated chronic inflammation [20].

Vitamin E, a vital fat-soluble antioxidant, plays a key role in the body's defense against oxidative stress. It functions primarily by protecting cell membranes from lipid peroxidation caused by ROS [21]. As a result, plasma levels of Vitamin E can serve as a reflection of the body's overall antioxidant capacity [22]. Moreover, Vitamin E is not just a passive protector; it also possesses powerful anti-inflammatory properties, making it a critical part of the body's anti-inflammatory arsenal [23]. A deficiency in Vitamin E can thus compromise the body's ability to combat both oxidative stress and inflammation, potentially exacerbating the symptoms and progression of PCOS [24]. The relationship between inflammatory markers like CRP and antioxidant levels like Vitamin E is therefore of great interest in understanding the disease [25].

The current study was designed to investigate the inflammatory status and antioxidant capacity of women newly diagnosed with PCOS [26]. By focusing on a cohort of untreated individuals, this research aimed to establish a clear baseline of CRP and Vitamin E levels before the confounding effects of therapeutic interventions could be introduced [27]. This case-control study compared 88 women with PCOS to 88 healthy, age-matched women to determine if significant differences existed in their serum CRP and Vitamin E concentrations [28, 29]. The central hypothesis was that women with PCOS would exhibit higher levels of inflammatory markers (CRP) and lower levels of anti-inflammatory markers (Vitamin E) compared to the healthy control group, reflecting an imbalance between the inflammatory and anti-inflammatory status [30].

Material and Methods

This was a hospital based case control study, newly diagnosed and clinically confirmed cases of PCOS from Obstetrics and Gynaecology Department in collaboration with department of biochemistry from Tertiary Care Hospital, in Pune were included for the study.

88 women of age group18-45 who were newly diagnosed with PCOS (according to Rotterdam's criteria) were taken as cases. 88 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. BJGMC/IEC/Pharmac/Ph.D./-0422049-049, 25-04-2022). Written informed consent was taken from all participants and obtained information was kept confidential and used only for the purpose of the study.

Patients with history of inflammatory diseases or on anti-inflammatory drugs, pregnant women, history of hypertension, diabetes mellitus, coronary artery disease, endocrine abnormalities, infection or inflammation were excluded.

Sample collection method

5 ml of blood sample was withdrawn from mid-cubital or anti-cubital vein under aseptic conditions and transferred into the plain tubes. One hour after collection, sample tubes were centrifuged at 3000rpm for 5 minutes. Obtained serum was separated and stored at 0-4 degree until the test. Two biochemical parameters were estimated, C-reactive Protein (CRP) by CRP-Turbiltex quantitative turbidimetric method (Spinreact kit) and Vitamin E by Genlisa TM Elisa kit method.

Statistical analysis was done by using statistical software package SPSS, version 26.0. IBM.

Observation and results

PCOS and control group comparison was done using "t" test.

Normality of numerical data was checked using Shapiro-Wilk test & was found that the data did not follow a normal curve; or for graded data, hence non-parametric tests have been used for comparisons.

Inter group comparison (2 groups) was done using Mann Whitney U test. Independent t test was carried out to compare quantitative parameters among different. P value < 0.05 was considered as threshold for statistical significance.

The statistical analysis was performed on two variables, C-reactive protein (CRP) levels, and Vitamin E levels. A significance level (alpha) of 0.05 was established a priori, meaning that a p-value less than 0.05 would be considered statistically significant.

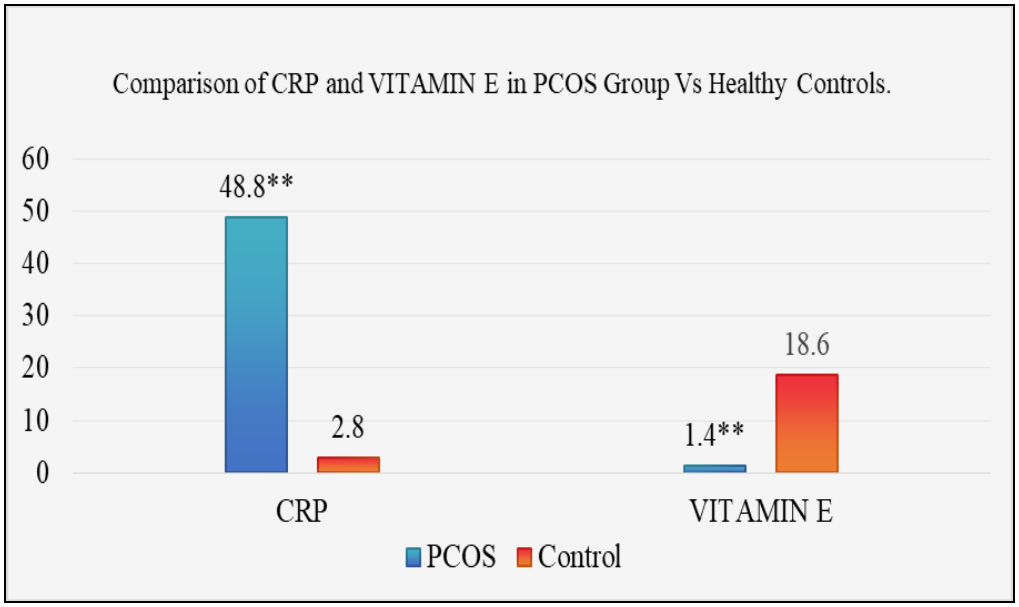
Mann-Whitney U test; **indicates statistically highly significant $p<0.01$; # indicates statistically non-significant $p>0.05$.

Graph no.1 showed that, there was a statistically highly significant difference seen ($p<0.01$) for CRP with higher values in PCOS group and Vitamin E between the pcos group and Control group, with higher values in controls.

Discussion

Our study showed significant increase in CRP level among PCOS women with a P value less than 0.001. 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. In another study done by Mohamadin *et al*. in 50 Saudi women with PCOS diagnosed using Rotterdam's criteria and found that a highly elevated hs-CRP level existed in PCOS patients [26].

Polycystic Ovary Syndrome (PCOS) subjects have higher levels of inflammation than healthy individuals of the same age.



Graph 1: Group Pair wise comparison of PCOS Group vs control for CRP and Vitamin E

This inflammation, as shown by elevated C-reactive protein (CRP), is also linked to other metabolic issues like high fasting insulin, microalbuminuria, and impaired fibrinolytic activity. CRP's role in the development of atherosclerosis is due to its effect on blood vessel lining and muscle cells, where it promotes the release of endothelin-1 and increases the presence of adhesion molecules and chemokines [27].

The evaluation of C-reactive protein (CRP) is essential for understanding the inflammatory status of the participants. A significant difference in CRP levels between the PCOS and control groups ($p<0.05$) is a powerful indicator of a chronic, low-grade inflammatory state in women with PCOS. Numerous studies have established this link, showing that women with PCOS often have higher CRP levels, which contributes to metabolic and cardiovascular complications such as insulin resistance and heart disease [28-30].

Furthermore, the assessment of Vitamin E levels provides a complementary perspective. Vitamin E is a potent antioxidant, but it also has a notable anti-inflammatory role, which is particularly relevant in conditions like PCOS. It works by modulating inflammatory pathways and suppressing the production of pro-inflammatory cytokines, which are signaling molecules that drive inflammation [29]. A statistically significant finding that the PCOS group has lower levels of Vitamin E ($p<0.05$) compared to the control group would suggest a diminished capacity to control inflammation. This deficiency in Vitamin E could compromise the body's natural anti-inflammatory defenses, contributing to the persistent low-grade inflammation observed in the condition. The findings demonstrate a highly significant increase in C-Reactive Protein (CRP) in women with PCOS ($p<0.001$), confirming a state of chronic inflammation that is linked to metabolic issues like high fasting insulin and cardiovascular risks. This persistent inflammation is further compounded by a suggested deficiency in Vitamin E ($p<0.05$). Lower Vitamin E levels indicate a diminished capacity for anti-inflammatory defense, compromising the body's natural ability to suppress the inflammatory pathways that drive the condition. In conclusion, the study strongly suggests that PCOS is a multifaceted disorder of both chronic inflammation and compromised anti-inflammatory response, highlighting the need for comprehensive treatment strategies that target these underlying inflammatory processes.

To summarize, the findings indicate significant differences in both CRP and Vitamin E levels, it would reinforce the understanding of PCOS as a multifaceted disorder characterized by both chronic inflammation and a weakened anti-inflammatory response. These insights are crucial for developing comprehensive treatment strategies that not only manage hormonal symptoms but also address the underlying inflammatory processes.

Conclusion

The findings of this study have significant implications for the clinical management of PCOS. This will help clinician to treat patients with vitamin E & manage dose of vitamin E supplementation. This could lead to a paradigm shift in how PCOS is treated, moving beyond traditional hormonal, for instance, it may highlight the potential benefit of adjunctive therapies such as antioxidant supplementation with Vitamin E, as a means to mitigate the inflammatory burden and improve patient outcomes. Ultimately, this research aims to contribute to a deeper and more comprehensive understanding of PCOS pathophysiology, paving the way for more effective, personalized, and holistic treatment plans that address the root causes of this widespread and debilitating condition.

Conflict of Interest

Not available

Financial Support

Not available

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