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Original Research Article

Evaluation of C-Reactive Protein Levels in Women Diagnosed with Polycystic Ovarian Syndrome on Metformin Therapy

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Abstract

Background: Polycystic ovarian syndrome (PCOS) is associated with chronic low-grade inflammation, with elevated C-reactive protein (CRP) levels being a key inflammatory marker. Metformin, commonly used in PCOS management, may have anti-inflammatory properties. This study evaluated the long-term impact of metformin therapy on CRP levels in women with PCOS over a one-year period.

Methods: A prospective observational study was conducted on 186 women diagnosed with PCOS according to Rotterdam criteria. Participants were initiated on metformin 500mg twice daily and followed for 12 months. Serum CRP levels were measured at baseline, 3 months, 6 months, 9 months, and 12 months using high-sensitivity CRP assay. Additional parameters including BMI, waist circumference, fasting glucose, insulin levels, and hormonal profiles were assessed. Statistical analysis was performed using SPSS version 25.0.

Results: The mean age of participants was 25.2 ± 4.6 years. Baseline CRP levels were significantly elevated (4.6 ± 2.1 mg/L) compared to normal reference values. Following metformin therapy, CRP levels decreased progressively: 3 months (3.1 ± 1.6 mg/L, $p<0.001$), 6 months (2.4 ± 1.3 mg/L, $p<0.001$), 9 months (2.0 ± 1.1 mg/L, $p<0.001$), and 12 months (1.7 ± 1.0 mg/L, $p<0.001$). The reduction in CRP levels correlated positively with improvements in insulin resistance ($r=0.689$, $p<0.001$) and BMI reduction ($r=0.591$, $p<0.001$). Maximum therapeutic benefit was observed after 9 months of treatment.

Conclusion: Long-term metformin therapy significantly reduces CRP levels in women with PCOS, with progressive improvement over 12 months. The sustained reduction in inflammatory markers suggests metformin's potential role in reducing long-term cardiovascular risk in PCOS patients.

Keywords: Polycystic ovarian syndrome, C-reactive protein, metformin, inflammation, insulin resistance, long-term therapy.

Introduction

Polycystic ovarian syndrome (PCOS) represents one of the most common endocrine disorders affecting women of reproductive age, with a prevalence ranging from 5% to 20% globally depending on the diagnostic criteria used [1].

The syndrome is characterized by a complex interplay of hormonal, metabolic, and inflammatory abnormalities that contribute to its diverse clinical manifestations including irregular menstrual cycles, hyperandrogenism, and polycystic ovarian morphology [2]. Beyond its reproductive implications, PCOS is increasingly recognized as a systemic condition with significant long-term health consequences, including increased risks of type 2 diabetes mellitus, cardiovascular disease, metabolic syndrome, and endometrial cancer [3]. The pathophysiology of PCOS involves multiple interconnected mechanisms, with insulin resistance being considered a central feature affecting approximately 65-80% of women with the condition [4].

Insulin resistance in PCOS is accompanied by compensatory hyperinsulinemia, which stimulates androgen production from both ovarian theca cells and adrenal glands, thereby perpetuating the hormonal imbalances characteristic of the syndrome. This insulin resistance is often independent of obesity, although the coexistence of excess weight can exacerbate the metabolic dysfunction and worsen the clinical presentation. The prevalence of insulin resistance in PCOS patients varies significantly based on BMI, with

rates of 75-95% in obese women compared to 30-50% in lean women with the condition [5].

Emerging evidence suggests that chronic low-grade inflammation plays a crucial role in the development and progression of PCOS. This inflammatory state is characterized by elevated levels of various inflammatory markers, including C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-18 (IL-18), and nuclear factor-kappa B (NF-κB) [6]. Among these markers, CRP has gained particular attention due to its stability, standardized measurement methods, established association with cardiovascular risk, and relatively low cost of measurement. CRP is an acute-phase reactant synthesized primarily by hepatocytes in response to inflammatory cytokines, particularly IL-6, and serves as a sensitive marker of systemic inflammation with established predictive value for cardiovascular events [7].

Multiple studies have demonstrated that women with PCOS exhibit significantly higher CRP levels compared to healthy controls, even after adjusting for body mass index (BMI) and other confounding factors. The elevation in CRP levels appears to be present across different PCOS phenotypes and is observed in both lean and obese women with the condition, suggesting that the inflammatory state is intrinsic to the syndrome rather than merely a consequence of obesity. Meta-analyses have reported that CRP levels in PCOS patients are typically 2-3 times higher than in healthy controls, with mean differences ranging from 1.5 to 3.0 mg/L [8]. The chronic inflammatory environment in PCOS may contribute to insulin resistance through various mechanisms, including interference with insulin signaling pathways, promotion of oxidative stress, alteration of adipokine production, and activation of inflammatory cascades involving toll-like receptors [9].

The relationship between inflammation and insulin resistance in PCOS creates a vicious cycle where each component reinforces the other. Elevated insulin levels can stimulate the production of inflammatory cytokines through multiple pathways, including activation of NF-κB and increased production of advanced glycation end products. Conversely, the inflammatory state can worsen insulin resistance through direct effects on insulin signaling pathways, particularly through phosphorylation of insulin receptor substrate-1 (IRS-1) at serine residues, which reduces insulin sensitivity. This interconnection has important therapeutic implications, as interventions targeting either inflammation or insulin resistance may have beneficial effects on both components of this pathophysiological cycle [10]. Metformin, a biguanide derivative, has emerged as a cornerstone therapy in the management of PCOS, particularly for addressing the metabolic aspects of the syndrome. Originally developed as an antidiabetic medication, metformin's mechanism of action involves activation of adenosine monophosphate-activated protein kinase (AMPK), leading to decreased hepatic glucose production, improved peripheral glucose uptake, and enhanced insulin sensitivity. The activation of AMPK also results in multiple downstream effects including increased fatty acid oxidation, reduced lipogenesis, and modulation of inflammatory pathways. In the context of PCOS, metformin has been shown to improve insulin resistance, reduce androgen levels, restore ovulatory cycles, improve metabolic parameters including lipid profiles and blood pressure, and potentially reduce the risk of gestational diabetes in pregnancy. Long-term studies have suggested that metformin therapy may also reduce the risk of developing type 2 diabetes mellitus in women with PCOS, although the evidence for cardiovascular protection remains limited.

Beyond its well-established metabolic effects, mounting evidence suggests that metformin may possess significant anti-inflammatory properties that could contribute to its therapeutic benefits in PCOS. Several studies have reported that metformin therapy can reduce levels of inflammatory markers, including CRP, TNF-α, IL-6, and IL-18, in various patient populations including those with diabetes, metabolic syndrome, and cardiovascular disease. The anti-inflammatory effects of metformin appear to be mediated through multiple mechanisms, including direct inhibition of NF-κB activation, reduction in oxidative stress through enhancement of antioxidant enzyme activity, modulation of immune cell function, and improvement in endothelial function. Additionally, metformin has been shown to reduce the production of advanced glycation end products and their inflammatory effects, which may be particularly relevant in the context of PCOS-associated insulin resistance. However, the specific impact of metformin on CRP levels in women with PCOS remains incompletely characterized, with existing studies showing variable results and methodological limitations. Most previous studies have been limited by small sample sizes, short follow-up periods, heterogeneous study populations, and lack of standardized treatment protocols. Furthermore, the time course of metformin's anti-inflammatory effects and the relationship between duration of therapy and magnitude of CRP reduction have not been well established. Understanding these relationships is crucial for optimizing treatment strategies and predicting long-term outcomes in women with PCOS.

Aims and Objectives: The primary aim of this study was to evaluate the long-term effect of metformin therapy on C-reactive protein levels in women diagnosed with polycystic ovarian syndrome over a twelve-month treatment period. The study sought to determine whether metformin therapy resulted in significant and sustained changes in CRP levels and to characterize the time course of these changes throughout the treatment period. The secondary objectives included comprehensive assessment of the correlation between changes in CRP levels and improvements in other metabolic parameters, including insulin resistance indices, homeostatic model assessment of insulin resistance (HOMA-IR), body mass index, waist circumference, and hormonal profiles including testosterone and sex hormone-binding globulin levels. The study also aimed to identify baseline characteristics that might predict the response to metformin therapy in terms of CRP reduction, including age, BMI, initial CRP levels, and degree of insulin resistance. Additionally, the research investigated the relationship between the duration of metformin therapy and the degree of CRP reduction, examining whether the anti-inflammatory effects were time-dependent and progressive throughout the treatment period. The study also sought to determine the optimal duration of treatment for achieving maximum anti-inflammatory benefits and to assess the sustainability of these effects over the one-year follow-up period.

Materials and Methods

Study Design and Setting: This prospective observational study was conducted at the Department of Obstetrics and Gynecology of a tertiary care centre in Karnataka, India, between January 2022 and February 2024. The study protocol was approved by the Institutional Ethics Committee (IEC approval number: SIMS/IEC/2022/001), and written informed consent was obtained from all participants prior to enrollment. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines.

Study Population and Sample Size: The study population consisted of women aged 18-35 years diagnosed with PCOS according to the Rotterdam criteria (2003), which required the presence of at least two of the following three features: oligo-ovulation or anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries on ultrasound examination. The sample size was calculated based on previous studies reporting CRP levels in PCOS patients, with an expected mean difference of 2.0 mg/L in CRP levels before and after treatment, standard deviation of 2.5 mg/L, power of 90%, and significance level of 0.05. Using these parameters, the minimum required sample size was determined to be 165 participants. Accounting for a 15% dropout rate over the one-year follow-up period, a total of 195 women were initially screened, with 186 meeting the inclusion criteria and being enrolled in the study.

Inclusion and Exclusion Criteria

Inclusion criteria comprised women aged 18-35 years with confirmed diagnosis of PCOS according to Rotterdam criteria, BMI between 18.5-40 kg/m², elevated baseline CRP levels (>1.0 mg/L), no history of metformin therapy in the preceding six months, and willingness to comply with the study protocol and follow-up visits for the entire one-year period. Participants were required to have stable lifestyle patterns and no plans for major dietary changes or weight loss interventions during the study period.

Exclusion criteria included pregnancy or lactation, presence of diabetes mellitus or other endocrine disorders including thyroid dysfunction and Cushing's syndrome, active inflammatory conditions or infections, autoimmune diseases, cardiovascular disease, liver dysfunction (ALT/AST >2 times upper limit of normal), kidney dysfunction (serum creatinine >1.2 mg/dL), malignancy, use of anti-inflammatory medications or hormonal therapies within the previous six months, contraindications to metformin therapy including history of lactic acidosis, and concurrent use of medications known to affect CRP levels. Women with recent surgery or trauma within the preceding two months were also excluded from the study.

Intervention Protocol: All eligible participants were initiated on metformin therapy at a dose of 500 mg twice daily with meals. The medication was provided by the hospital pharmacy as extended-release tablets to improve tolerance and compliance. Participants were started on 500 mg once daily for the first week, followed by 500 mg twice daily from the second week onwards to minimize gastrointestinal side effects. The medication was provided free of charge to ensure compliance, and participants were counseled about potential side effects including gastrointestinal disturbances, metallic taste, and rare risk of lactic acidosis. Participants were advised to report any adverse events promptly and were provided with 24-hour contact information for medical emergencies.

Clinical Assessment and Data Collection: Comprehensive clinical assessment was performed at baseline, 3 months, 6 months, 9 months, and 12 months. Demographic data, medical history, family history, and clinical examination findings were recorded using standardized case report forms. Anthropometric measurements including height,

weight, BMI, waist circumference, hip circumference, and waist-to-hip ratio were obtained using calibrated instruments following standard protocols. Blood pressure was measured using a standard sphygmomanometer after 10 minutes of rest in seated position, with the average of three readings recorded. Medication compliance was assessed through pill counts, patient diaries, and direct questioning at each visit.

Laboratory Investigations: Venous blood samples were collected after 12 hours of fasting at baseline, 3 months, 6 months, 9 months, and 12 months. All samples were collected between 8:00 AM and 10:00 AM to minimize circadian variations. Serum CRP levels were measured using high-sensitivity CRP assay (hsCRP) with chemiluminescent immunoassay technology on Abbott Architect i2000SR analyzer. The assay had a lower detection limit of 0.1 mg/L, upper limit of 20 mg/L, and coefficient of variation of less than 5%. Quality control was maintained through daily calibration and internal quality control samples.

Additional biochemical parameters included fasting glucose measured by glucose oxidase method, insulin levels by electrochemiluminescence immunoassay, glycated hemoglobin (HbA1c) by high-performance liquid chromatography, and lipid profile including total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol. Liver function tests including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum creatinine were monitored for safety. Insulin resistance was calculated using the homeostatic model assessment of insulin resistance (HOMA-IR) formula: (fasting insulin × fasting glucose)/22.5.

Hormonal assessment included total testosterone by electrochemiluminescence immunoassay, sex hormone-binding globulin (SHBG) by immunoradiometric assay, dehydroepiandrosterone sulfate (DHEAS) by chemiluminescent immunoassay, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin levels. Free androgen index (FAI) was calculated as (total testosterone × 100)/SHBG. All hormonal assessments were performed during the follicular phase of the menstrual cycle or on random days for women with amenorrhea.

Statistical Analysis: Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as mean ± standard deviation for continuous variables and frequencies with percentages for categorical variables. Normality of data distribution was assessed using the Shapiro-Wilk test. Repeated measures analysis of variance (ANOVA) was used to compare CRP levels and other continuous variables across different time points. Post-hoc analysis was performed using Bonferroni correction for multiple comparisons. Pearson correlation analysis was used to assess relationships between changes in CRP levels and other variables. Linear regression analysis was performed to identify predictors of CRP reduction. A p-value of <0.05 was considered statistically significant.

Results

Baseline Characteristics: A total of 186 women with PCOS were enrolled in the study, with 174 participants completing the full 12-month follow-up period, representing a dropout rate of 6.5%. The mean age of participants was 25.2 ± 4.6 years, with the majority (78.5%) being in the age group of 20-30 years. The mean BMI was 27.8 ± 5.2 kg/m², with 42.3% of participants classified as overweight and 35.5% as obese. The mean duration of PCOS symptoms was 3.8 ± 2.1 years, and 68.8% of participants presented with irregular menstrual cycles as the primary complaint.

C-Reactive Protein Levels: Baseline CRP levels were significantly elevated in the study population with a mean value of 4.6 ± 2.1 mg/L, which was considerably higher than the normal reference range (<1.0 mg/L for low cardiovascular risk). Following initiation of metformin therapy, there was a progressive and statistically significant reduction in CRP levels at all time points compared to baseline.

At 3 months, the mean CRP level decreased to 3.1 ± 1.6 mg/L ($p<0.001$), representing a 32.6% reduction from baseline. This reduction continued at 6 months with a mean CRP level of 2.4 ± 1.3 mg/L ($p<0.001$), indicating a 47.8% reduction from baseline. Further improvement was observed at 9 months with a mean CRP level of 2.0 ± 1.1 mg/L ($p<0.001$), representing a 56.5% reduction from baseline. The maximum reduction was achieved at 12 months with a mean CRP level of 1.7 ± 1.0 mg/L ($p<0.001$), indicating a 63.0% reduction from baseline values.

Metabolic Parameters: Significant improvements were observed in various metabolic parameters throughout the study period. The mean BMI decreased from 27.8 ± 5.2 kg/m² at baseline to 26.1 ± 4.8 kg/m² at 12 months ($p<0.001$). Waist circumference showed a reduction from 89.4 ± 12.3 cm at baseline to 84.7 ± 11.2 cm at 12 months ($p<0.001$). Fasting glucose levels decreased from 96.8 ± 14.2 mg/dL at baseline to 88.3 ± 11.8 mg/dL at 12 months ($p<0.001$). Insulin levels showed a significant reduction from 18.6 ± 8.4 μIU/mL at baseline to 12.8 ± 5.9 μIU/mL at 12 months ($p<0.001$). The HOMA-IR index decreased from 4.5 ± 2.1 at baseline to 2.8 ± 1.4 at 12 months ($p<0.001$).

Hormonal Parameters: Significant improvements were observed in hormonal profiles following metformin therapy. Total testosterone levels decreased from 68.4 ± 24.8 ng/dL at baseline to 54.2 ± 20.3 ng/dL at 12 months ($p<0.001$). Sex hormone-binding globulin levels increased from 32.6 ± 14.2 nmol/L at baseline to 42.8 ± 18.6 nmol/L at 12 months ($p<0.001$). The free androgen index decreased from 8.9 ± 4.2 at baseline to 6.1 ± 3.1 at 12 months ($p<0.001$). Dehydroepiandrosterone sulfate levels showed a reduction from 286.4 ± 98.7 µg/dL at baseline to 234.8 ± 82.3 µg/dL at 12 months ($p<0.001$).

Correlation Analysis: Strong positive correlations were observed between the reduction in CRP levels and improvements in various metabolic parameters. The correlation between CRP reduction and improvement in HOMA-IR was particularly strong ($r=0.689$, $p<0.001$).

A significant correlation was also observed between CRP reduction and BMI improvement ($r=0.591$, $p<0.001$). The correlation between CRP reduction and testosterone level improvement was moderate ($r=0.445$, $p<0.001$). Additionally, a significant correlation was found between baseline CRP levels and the magnitude of CRP reduction ($r=0.612$, $p<0.001$), indicating that participants with higher baseline CRP levels experienced greater absolute reductions.

Predictors of CRP Response: Linear regression analysis identified several baseline characteristics as significant predictors of CRP reduction. Baseline CRP levels were the strongest predictor ($\beta=0.624$, $p<0.001$), followed by baseline HOMA-IR ($\beta=0.382$, $p<0.001$) and baseline BMI ($\beta=0.298$, $p<0.001$).

Age showed a weak but significant negative correlation with CRP reduction ($\beta=-0.156$, $p=0.023$), suggesting that younger participants had better responses to treatment.

Table 1: Baseline Characteristics of Study Participants (n=186)

Parameter	Mean ± SD / n (%)
Age (years)	25.2 ± 4.6
BMI (kg/m²)	27.8 ± 5.2
Waist circumference (cm)	89.4 ± 12.3
Duration of PCOS symptoms (years)	3.8 ± 2.1
Irregular menstrual cycles	128 (68.8%)
Hirsutism	112 (60.2%)
Acne	94 (50.5%)
Family history of diabetes	78 (41.9%)
Family history of PCOS	45 (24.2%)
Oligomenorrhea	142 (76.3%)
Amenorrhea	44 (23.7%)

Table 2: Changes in C-Reactive Protein Levels Over 12 Months (n=174)

Time Point	CRP (mg/L)	% Change from Baseline	p-value vs Baseline
Baseline	4.6 ± 2.1	-	-
3 months	3.1 ± 1.6	-32.6%	<0.001
6 months	2.4 ± 1.3	-47.8%	<0.001
9 months	2.0 ± 1.1	-56.5%	<0.001
12 months	1.7 ± 1.0	-63.0%	<0.001

Table 3: Changes in Metabolic Parameters Over 12 Months (n=174)

Parameter	Baseline	6 months	12 months	p-value
BMI (kg/m²)	27.8 ± 5.2	26.9 ± 4.9	26.1 ± 4.8	<0.001
Waist circumference (cm)	89.4 ± 12.3	86.8 ± 11.7	84.7 ± 11.2	<0.001
Fasting glucose (mg/dL)	96.8 ± 14.2	92.1 ± 12.6	88.3 ± 11.8	<0.001
Insulin (µIU/mL)	18.6 ± 8.4	15.2 ± 7.1	12.8 ± 5.9	<0.001
HOMA-IR	4.5 ± 2.1	3.5 ± 1.8	2.8 ± 1.4	<0.001
Total cholesterol (mg/dL)	189.4 ± 32.6	176.8 ± 28.9	168.3 ± 26.4	<0.001
Triglycerides (mg/dL)	142.8 ± 48.3	128.6 ± 42.1	118.9 ± 38.7	<0.001

Table 4: Changes in Hormonal Parameters Over 12 Months (n=174)

Parameter	Baseline	6 months	12 months	p-value
Total testosterone (ng/dL)	68.4 ± 24.8	61.2 ± 22.4	54.2 ± 20.3	<0.001
SHBG (nmol/L)	32.6 ± 14.2	37.8 ± 16.4	42.8 ± 18.6	<0.001
Free androgen index	8.9 ± 4.2	7.4 ± 3.8	6.1 ± 3.1	<0.001
DHEAS (μg/dL)	286.4 ± 98.7	258.6 ± 89.2	234.8 ± 82.3	<0.001
LH (mIU/mL)	12.8 ± 5.4	10.6 ± 4.8	9.2 ± 4.2	<0.001
FSH (mIU/mL)	5.8 ± 2.1	6.2 ± 2.3	6.4 ± 2.4	0.082
LH/FSH ratio	2.3 ± 0.9	1.8 ± 0.7	1.5 ± 0.6	<0.001

Table 5: Correlation Analysis Between CRP Reduction And Other Parameters

Parameter	Correlation Coefficient (r)	p-value
HOMA-IR improvement	0.689	<0.001
BMI reduction	0.591	<0.001
Waist circumference reduction	0.534	<0.001
Testosterone reduction	0.445	<0.001
Insulin reduction	0.612	<0.001
Triglyceride reduction	0.398	<0.001
SHBG increase	-0.367	<0.001
Weight reduction	0.478	<0.001

Table 6: Predictors of CRP Response - Linear Regression Analysis

Predictor	Beta Coefficient	Standard Error	p-value	95% CI
Baseline CRP	0.624	0.048	<0.001	0.529-0.719
Baseline HOMA-IR	0.382	0.067	<0.001	0.249-0.515
Baseline BMI	0.298	0.089	<0.001	0.122-0.474
Age	-0.156	0.068	0.023	-0.290--0.022
Duration of PCOS	0.089	0.072	0.218	-0.053-0.231
Baseline testosterone	0.134	0.074	0.071	-0.012-0.280

Discussion

The present study demonstrates that metformin therapy produces significant and sustained reductions in C-reactive protein levels in women with PCOS over a 12-month treatment period. The progressive decrease in CRP levels, with maximum benefit achieved at 12 months, suggests that the anti-inflammatory effects of metformin are both dose-dependent and time-dependent, requiring sustained therapy for optimal therapeutic benefit. These findings have important implications for understanding the mechanisms of metformin action in PCOS and its potential role in reducing long-term cardiovascular risk in this population.

The baseline CRP levels observed in this study (4.6 ± 2.1 mg/L) were consistent with previous reports demonstrating elevated inflammatory markers in women with PCOS [11]. A meta-analysis by Escobar-Morreale et al. reported that CRP levels in PCOS patients were typically 2-3 times higher than in healthy controls, with mean levels ranging from 3.2 to 5.8 mg/L [12]. The elevated CRP levels in PCOS patients are clinically significant, as levels above 3.0 mg/L are associated with high cardiovascular risk according to established guidelines. The progressive reduction in CRP levels observed in this study, with a final reduction of 63.0% at 12 months, represents a clinically meaningful improvement that could translate into reduced cardiovascular risk.

The time course of CRP reduction observed in this study provides valuable insights into the optimal duration of metformin therapy. While significant reductions were observed as early as 3 months, the maximum benefit was achieved at 12 months, suggesting that prolonged therapy is required for optimal anti-inflammatory effects. This finding is consistent with previous studies demonstrating that metformin's metabolic effects also improve progressively over time, with maximum benefits typically observed after 6-12 months of therapy [13]. The continued improvement in CRP levels beyond 6 months suggests that clinicians should consider long-term metformin therapy for women with PCOS to achieve maximum anti-inflammatory benefits.

The strong correlation between CRP reduction and improvement in insulin resistance ($r=0.689$, $p<0.001$) supports the hypothesis that metformin's anti-inflammatory effects are closely linked to its metabolic actions. This finding is consistent with the concept that inflammation and insulin resistance form a vicious cycle in PCOS, where improvement in one component leads to improvement in the other [14]. The mechanistic relationship between metformin's effects on

inflammation and insulin resistance likely involves multiple pathways, including direct inhibition of inflammatory cascades, improvement in endothelial function, and modulation of adipokine production.

Comparison with previous studies reveals both similarities and differences in the magnitude of CRP reduction achieved with metformin therapy. A study by Morin-Papunen et al. reported a 40% reduction in CRP levels after 6 months of metformin therapy in women with PCOS, which is consistent with the 47.8% reduction observed in this study at the same time point [15]. However, longer-term studies with 12-month follow-up are limited, making the current findings particularly valuable for understanding the sustained effects of metformin therapy. The greater magnitude of CRP reduction observed in this study compared to some previous reports may be attributed to the larger sample size, longer follow-up period, and standardized treatment protocol.

The correlation between baseline CRP levels and the magnitude of CRP reduction ($r=0.612$, $p<0.001$) suggests that women with higher baseline inflammatory markers may derive greater benefit from metformin therapy. This finding has important clinical implications, as it suggests that CRP levels could potentially be used as a biomarker to predict treatment response and guide therapeutic decisions. Women with markedly elevated CRP levels may be prioritized for metformin therapy, while those with lower baseline levels may require different therapeutic approaches or additional interventions.

The improvements in hormonal parameters observed in this study, including reductions in testosterone levels and increases in SHBG, are consistent with previous reports and support the multifaceted benefits of metformin therapy in PCOS [16]. The moderate correlation between CRP reduction and testosterone improvement ($r=0.445$, $p<0.001$) suggests that the anti-inflammatory effects of metformin may contribute to its beneficial effects on hyperandrogenism. This relationship is biologically plausible, as inflammatory cytokines can stimulate androgen production through various mechanisms, including activation of steroidogenic enzymes and enhancement of luteinizing hormone action. The safety profile of metformin therapy in this study was excellent, with only minor gastrointestinal side effects reported in 12.4% of participants during the initial weeks of treatment, which resolved with continued therapy. No cases of lactic acidosis or significant liver dysfunction were observed, confirming the safety of long-term metformin therapy in this population. The low dropout rate of 6.5% over 12 months demonstrates good tolerability and patient acceptance of the treatment regimen.

However, this study has several limitations that should be acknowledged. The observational design without a control group limits the ability to establish causality between metformin therapy and CRP reduction. While the progressive nature of the improvement and strong correlations with metabolic parameters support a causal relationship, randomized controlled trials would provide stronger evidence. Additionally, the study population was limited to a single center in South India, which may limit the generalizability of findings to other populations with different genetic backgrounds, dietary patterns, and lifestyle factors.

The exclusion of women with diabetes mellitus may have resulted in a study population with milder metabolic dysfunction compared to the general PCOS population, potentially affecting the magnitude of treatment response. Furthermore, the study did not include measurement of other inflammatory markers such as TNF-α, IL-6, or IL-18, which could have provided additional insights into the anti-inflammatory mechanisms of metformin. The lack of assessment of lifestyle factors such as diet and physical activity, which can influence both CRP levels and treatment response, represents another limitation.

Despite these limitations, the study provides valuable evidence for the anti-inflammatory effects of metformin in PCOS and supports its role as a first-line therapy for women with this condition. The findings suggest that metformin therapy should be considered not only for its metabolic benefits but also for its potential to reduce chronic inflammation and associated cardiovascular risk. Future research should focus on randomized controlled trials with longer follow-up periods, inclusion of diverse populations, and investigation of optimal dosing strategies for achieving maximum anti-inflammatory benefits.

The clinical implications of these findings extend beyond the immediate management of PCOS symptoms. Given the association between elevated CRP levels and increased cardiovascular risk, the significant reduction in CRP levels achieved with metformin therapy may translate into reduced long-term cardiovascular morbidity and mortality in women with PCOS. This potential benefit supports the use of metformin as a preventive therapy in young women with PCOS, even in the absence of overt metabolic dysfunction or diabetes.

Conclusion

This prospective study demonstrates that metformin therapy produces significant, progressive, and sustained reductions in C-reactive protein levels in women with polycystic ovarian syndrome over a 12-month treatment period. The 63.0% reduction in CRP levels achieved at 12

months represents a clinically meaningful improvement that may translate into reduced cardiovascular risk. The strong correlation between CRP reduction and improvements in insulin resistance, body mass index, and hormonal parameters supports the interconnected nature of inflammation and metabolic dysfunction in PCOS.

The time-dependent nature of the anti-inflammatory effects, with maximum benefit achieved after 12 months of therapy, suggests that prolonged metformin treatment is required for optimal therapeutic outcomes. Women with higher baseline CRP levels demonstrated greater absolute reductions, indicating that inflammatory markers may serve as useful biomarkers for predicting treatment response. These findings support the use of metformin as a first-line therapy for women with PCOS, not only for its established metabolic benefits but also for its significant anti-inflammatory effects.

The sustained reduction in inflammatory markers observed with long-term metformin therapy may have important implications for reducing the long-term health risks associated with PCOS, including cardiovascular disease and type 2 diabetes mellitus. Future randomized controlled trials with larger sample sizes and longer follow-up periods are warranted to confirm these findings and establish optimal treatment protocols for achieving maximum anti-inflammatory benefits in women with PCOS.

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