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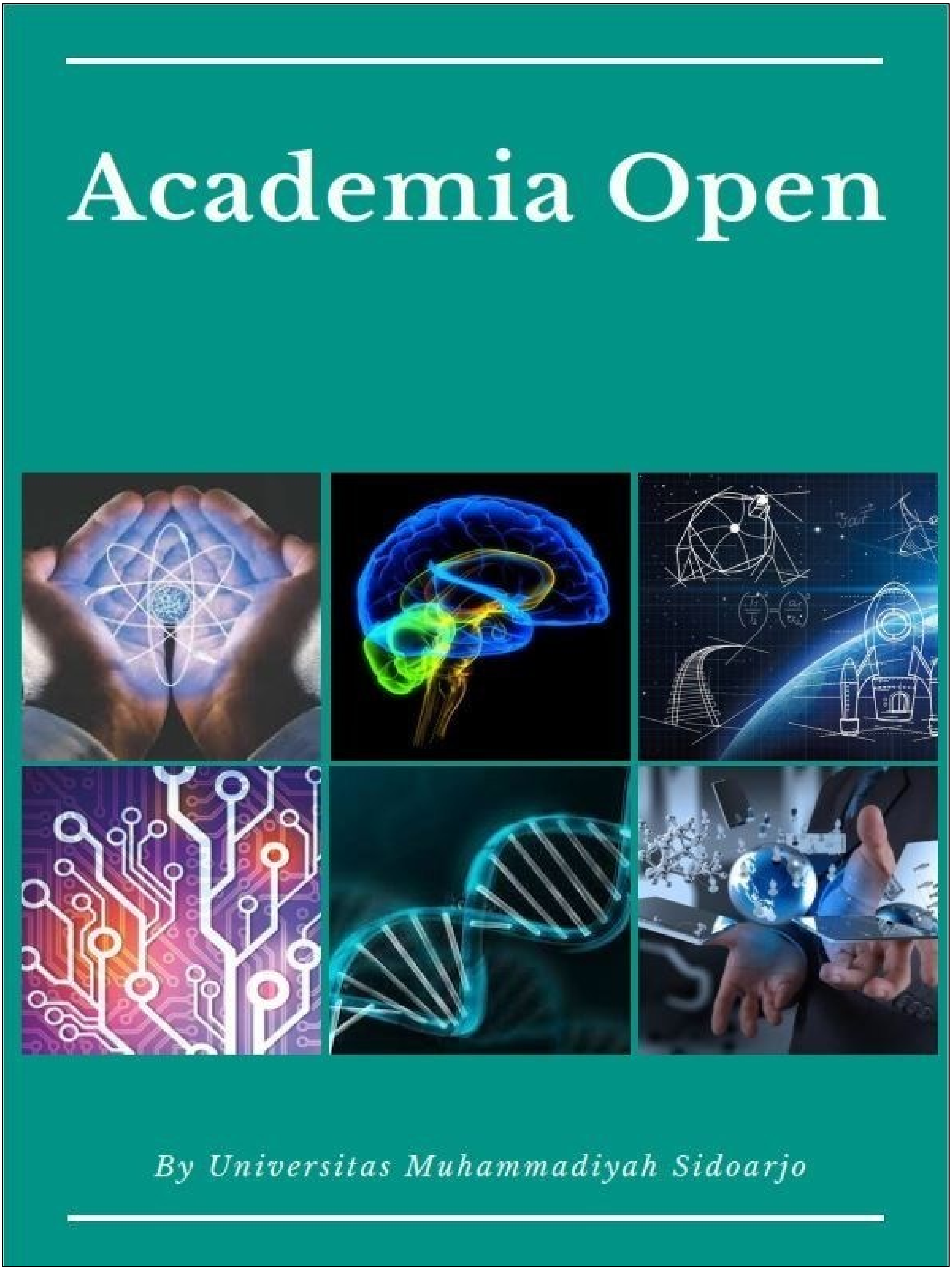

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Nimesulide with Metformin Lowers Inflammatory Markers in Women with PCOS

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Abstract

General Background: Polycystic Ovary Syndrome (PCOS) is a chronic endocrine and metabolic disorder characterized by hyperandrogenism, anovulation, and low-grade systemic inflammation, which contributes to insulin resistance and cardiovascular risk. **Specific Background:** Metformin, the first-line treatment for PCOS, has known anti-inflammatory properties, but whether its combination with selective COX-2 inhibitors such as nimesulide offers superior anti-inflammatory efficacy remains underexplored. **Knowledge Gap:** Evidence on adjunctive NSAID therapy targeting inflammation in PCOS is limited, and the combined effects of metformin and nimesulide on key inflammatory biomarkers, interleukin-6 (IL-6) and C-reactive protein (CRP), have not been systematically evaluated. **Aims:** This study investigated whether adding nimesulide to metformin enhances anti-inflammatory effects in women with PCOS. **Results:** In a non-randomized controlled study of 100 participants, both treatments significantly reduced IL-6 and CRP; however, combination therapy produced greater CRP reductions ($p < 0.001$), while IL-6 changes were comparable. Regression analysis identified total cholesterol and free testosterone as independent predictors of residual IL-6 levels ($R^2 = 0.516$). **Novelty:** This study is among the first to demonstrate synergistic anti-inflammatory effects of COX-2 inhibition with metformin in PCOS. **Implications:** Findings support the integration of metabolic and anti-inflammatory strategies for PCOS management and highlight the need for randomized trials assessing long-term efficacy and safety of adjunctive COX-2 inhibitors.

Highlights:

- Combination therapy with nimesulide enhances metformin's anti-inflammatory effect on CRP.
- IL-6 reduction was comparable between metformin alone and combination treatment.
- Total cholesterol and free testosterone independently predict residual inflammation.

Keywords: PCOS, Metformin, Nimesulide, Inflammation, C-Reactive Protein

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Introduction

Polycystic Ovary Syndrome (PCOS) is a common chronic endocrine and metabolic disorder affecting 6–20% of women of reproductive age, typically diagnosed using the Rotterdam criteria: hyperandrogenism,

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oligo/anovulation, and polycystic ovaries [1]. Beyond reproductive issues, PCOS contributes to long-term metabolic complications including insulin resistance (IR), dyslipidemia, and cardiovascular risk [2].

Recent studies establish chronic low-grade inflammation as a central pathophysiological feature of PCOS, with elevated cytokines like interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) contributing to IR and ovarian dysfunction [3] [4] [5]. Systemic inflammation is exacerbated by obesity and central fat accumulation, which is common in PCOS and positively correlates with IL-6 and IR [6]. Dysregulated cytokine ratios—e.g., TNF-α/IL-6—reflect immune imbalance [7], while even first-degree relatives of PCOS patients show elevated inflammatory markers and IR, independent of BMI [8]. Studies also show that anti-inflammatory therapies—such as IL-6-targeted drugs or agents like crocin—can improve metabolic and inflammatory profiles in PCOS [9] [10]. Inflammation not only drives PCOS pathogenesis but also represents a key therapeutic target to prevent its long-term metabolic consequences [11].

Metformin, a first-line treatment in polycystic ovary syndrome (PCOS), offers significant benefits beyond insulin sensitization. It exhibits anti-inflammatory actions by inhibiting NF-κB and reducing pro-inflammatory cytokines like IL-6 and TNF-α [12] [13] [14]. It also improves endothelial function by lowering adhesion molecules such as ICAM-1 and enhancing vascular health [15]. In pregnant women with PCOS, metformin alters cytokine profiles, suggesting immune modulation [16].

Nimesulide is a preferential COX-2 inhibitor with established anti-inflammatory and analgesic effects. It reduces IL-6 and TNF-α production and modulates immune responses beyond COX-2 inhibition [17] [18]. Emerging evidence suggests potential metabolic effects, though this remains underexplored in PCOS.

While metformin's anti-inflammatory profile is well-documented, few studies have examined its combined use with nonsteroidal anti-inflammatory drugs (NSAIDs) in PCOS. This gap is critical, especially given the potential complementary effects of COX-2 inhibition and AMPK activation pathways. The current study aims to evaluate whether a metformin-nimesulide combination can synergistically lower IL-6 and CRP levels in PCOS, providing a mechanistic rationale for combination therapy to target chronic inflammation more effectively.

The study was designed to evaluate inflammatory outcomes strictly reflected in four prespecified analyses derived from the Results chapter. It aimed to test, within the metformin group, whether interleukin-6 and C-reactive protein decreased from baseline to post-treatment using paired t-tests with the exact statistics reported in Table 1. It also aimed to quantify, within the nimesulide plus metformin group, the pre- to post-treatment changes in interleukin-6 and C-reactive protein, including mean differences, confidence intervals, t values, and effect sizes exactly as presented in Table 2. A further objective was to compare post-treatment interleukin-6 and C-reactive protein between the two groups using independent samples t-tests, employing the post-treatment means, standard deviations, t values, degrees of freedom, and p values reported in the unnumbered table. In addition, the study aimed to model interleukin-6 post-treatment with multiple linear regression exactly as specified in the IL-6_post regression table, treating the listed biochemical

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variables and group as predictors and evaluating their coefficients and statistical significance. Collectively, these objectives were limited to establishing within-group reductions, between-group post-treatment differences, and multivariable correlates of interleukin-6 using only the variables and inferential outputs contained in the four tables, without introducing endpoints or analyses beyond them.

Methods

A. Study Design and Setting

A prospective, non-randomized, controlled clinical study was conducted in hospital-based and outpatient clinics in Misan and Basrah. All visits, specimen collections, and laboratory procedures followed the thesis protocol. Each participant completed a 3-week intervention, with standardized baseline and end-of-treatment assessments.

B. Participants

1. Target Population and Recruitment

Women of reproductive age with a clinical diagnosis of PCOS were screened in participating clinics. Recruitment followed a structured workflow: screening interview → eligibility verification → baseline assessment → group allocation. Participants were informed, consented in a private setting, and allowed to withdraw at any time without consequence to medical care.

a. Inclusion Criteria

- Clinical diagnosis of PCOS based on accepted criteria (menstrual irregularity with clinical and/or biochemical hyperandrogenism).
- Clinically stable and able to complete all study procedures and visits.
- No exposure to hormonal or anti-inflammatory therapies within the prespecified wash-out period prior to baseline.

b. Exclusion Criteria

- Chronic systemic illness likely to confound outcomes or increase risk (e.g., diabetes mellitus; cardiovascular, hepatic, renal, or significant gastrointestinal disease).
- Endocrine disorders that mimic/overlap with PCOS (e.g., congenital adrenal hyperplasia, Cushing's syndrome, clinically relevant thyroid disease).
- History of hormone-sensitive malignancy or active gynecologic cancer.
- Known hypersensitivity to NSAIDs (including nimesulide).
- Pregnancy, lactation, or intention to conceive during the study.
- Current use of medications affecting metabolism or hormonal indices (e.g., systemic corticosteroids, antiepileptics, anticoagulants, hormonal contraceptives) or concomitant NSAIDs/COX-2 inhibitors.

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C. Sampling Strategy, Allocation, and Blinding

A purposive sampling approach was used to enroll eligible women. The study employed parallel groups without randomization or blinding, consistent with the thesis design.

1. Sample Size

The a priori calculation (two independent means; 95% confidence Z=1.96; 80% power Z=0.84; SD = 0.6; Δ = 0.5) yielded n ≈ 22.6 per group. Accounting for attrition and to preserve power, the per-group number was rounded up to 30. The final sample size was N = 100 participants, allocated as Metformin alone (n = 50) and Metformin + nimesulide (n =50).

2. Interventions

Participants were assigned to one of two arms for 3 consecutive weeks:

a. Control: Metformin alone (standard therapy).
b. Intervention: Metformin plus nimesulide 100 mg twice daily.

Adherence was supported by direct communication and scheduled follow-ups. Concomitant use of additional NSAIDs/COX-2 inhibitors was not permitted.

3. Outcomes and Assessment Schedule

This manuscript is limited to inflammatory outcomes reported in the thesis.

a. Primary outcomes: Change from baseline (pre-treatment) to week 3 (post-treatment) in interleukin-6 (IL-6, pg/mL) and C-reactive protein (CRP/hs-CRP, mg/L).
b. Safety outcomes: Adverse events (AEs), with particular attention to gastrointestinal and hepatic tolerability, captured per institutional procedures.
c. Prespecified covariates (for adjustment only): Triglycerides, HDL-cholesterol, total cholesterol. These are not outcomes in this paper to avoid overlap with the hormone-focused manuscript.

D. Data Collection Tools

Standardized, interviewer-administered forms captured demographics, medical history, and medication use. Clinical assessments were performed by trained staff under uniform procedures to minimize inter-observer variability.

1. Laboratory Procedures and Quality Control

a. Specimen handling: Venous blood collected into serum separator tubes by sterile venipuncture; centrifuged ~3,000 × g for 10 minutes; serum aliquoted and stored at −80 °C until analysis.
b. Assays:
   - IL-6: Quantified using a validated ELISA kit according to manufacturer instructions.
   - CRP/hs-CRP: Measured on the thesis laboratory's validated immunoassay platform.

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c. Quality assurance: Reagents equilibrated per protocol; calibration curves and controls run each batch; acceptance criteria followed the thesis QC plan; inter-assay variability maintained within predefined limits.

E. Data Management and Monitoring

All data were recorded in standardized study forms and transferred to electronic spreadsheets with verification checks. Outlier review followed predefined clinical and analytical plausibility criteria. AEs were documented and managed according to institutional protocols, with referral for further care when required. The thesis reports no imputation procedures (no missing primary outcome data requiring replacement).

F. Statistical Analysis

Data were summarized as mean ± standard deviation. All tests were two-tailed with $\alpha=0.05$, and exact p-values are reported where available. Analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, NY).

Within-group change: Pre- to post-treatment changes in inflammatory markers were evaluated using paired t-tests separately in each treatment arm. For the combination arm, results additionally included mean differences with 95% confidence intervals and Cohen's d computed from paired differences, as tabulated.

Between-group comparison: Independent-samples t-tests compared post-treatment IL-6 and CRP between groups. Levene's test assessed homogeneity of variances; t-tests were reported accordingly (equal-variances-assumed output with df consistent with n_1+n_2-2).

Multivariable modeling: Multiple linear regression was used to model IL-6_post as the dependent variable with the following prespecified predictors: LH_post, FSH_post, DHEAS_post, triglycerides_post, HDL_post, total cholesterol_post, free testosterone_post, CRP_post, and treatment group. Regression outputs included unstandardized coefficients (B), standard errors, standardized betas, t statistics, and p-values, alongside model fit indices (R, R^2, adjusted R^2, standard error of estimate) and omnibus ANOVA (F-test).

Sample size and handling: The a priori sample size was based on detecting a mean difference between two independent groups at 95% confidence and 80% power (assumed SD=0.6; $\Delta=0.5$). Analyses were conducted on complete cases; no imputation of primary outcomes was performed.

G. Ethical Considerations

The protocol (objectives, procedures, data handling, and safety monitoring) received approval from the thesis scientific and ethical review board. Written informed consent was obtained from every participant before any study procedure.

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Result and Discussion

A. Results

Table 1. Paired t-test Comparison of Pre- and Post-Treatment Values in the Metformin Group

Variable	Pre Mean ± SD	Post Mean ± SD	t-statistic	df	p-value
IL-6 (pg/mL)	5.70 ± 0.76	4.00 ± 0.73	21.79	48	<0.0001
CRP (mg/L)	7.24 ± 0.89	6.43 ± 0.73	14.79	48	<0.0001

Footnote. Paired t-test comparing within-group (pre vs post) changes in the Metformin group; two-tailed significance with $\alpha = 0.05$.

Description. In women receiving metformin alone for three weeks, both inflammatory mediators declined significantly from baseline. Interleukin-6 fell from 5.70 ± 0.76 pg/mL to 4.00 ± 0.73 pg/mL ($t = 21.79$, $df = 48$, $p < 0.0001$), and C-reactive protein decreased from 7.24 ± 0.89 mg/L to 6.43 ± 0.73 mg/L ($t = 14.79$, $df = 48$, $p < 0.0001$). These findings indicate a robust within-group anti-inflammatory effect associated with metformin over the short intervention period.

Table 2. Paired t-Test Results Comparing Pre- and Post-Treatment Measurements in the Nimesulide +

Variable	Pre Mean ± SD	Post Mean ± SD	Mean Difference	95% CI of Difference (Lower–Upper)	t-value	df	p-value (two-tailed)	Effect Size (Cohen's d)
IL-6 (pg/mL)	5.71 ± 1.00	3.68 ± 1.40	2.03	1.69 – 2.38	11.922	48	<0.001	1.703
CRP (mg/L)	7.20 ± 1.13	5.42 ± 0.87	1.78	1.51 – 2.05	13.285	48	<0.001	1.898

Footnote. Paired t-test comparing within-group (pre vs post) changes in the Nimesulide + Metformin group; two-tailed significance with $\alpha = 0.05$. Effect sizes are Cohen's d based on paired differences.

Description. In the combination arm, interleukin-6 declined from 5.71 ± 1.00 pg/mL to 3.68 ± 1.40 pg/mL, yielding a mean reduction of 2.03 pg/mL with a narrow 95% CI (1.69 to 2.38), a large t statistic (11.922), and a highly significant p-value (<0.001). C-reactive protein similarly fell from 7.20 ± 1.13 mg/L to 5.42 ± 0.87 mg/L, for a mean reduction of 1.78 mg/L (95% CI 1.51 to 2.05; $t = 13.285$; $p < 0.001$). The corresponding Cohen's d values (1.703 for IL-6 and 1.898 for CRP) indicate very large within-group effects over the three-week course.

Table 3. Independent Samples t-Test — Post-Treatment Comparison Between Metformin Alone vs. Nimesulide + Metformin (Inflammatory Markers Only)

Parameter	Group 1 Mean ± SD (Metformin; n=49)	Group 2 Mean ± SD (Combination; n=49)	Mean Difference	t-value	df	p-value (2-tailed)
IL6_post (pg/mL)	4.00 ± 0.73	3.68 ± 1.40	0.33	1.444	96	0.153

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CRP_post (mg/L)	6.43 ± 0.73	5.42 ± 0.87	1.00	6.208	96	<0.001

Footnote. Independent samples t-test comparing post-treatment values between groups; Levene's test applied as per chapter protocol; two-tailed $\alpha = 0.05$.

Description. Post-treatment interleukin-6 levels were numerically lower with combination therapy than with metformin alone, but the between-group difference did not reach statistical significance (mean difference 0.33 pg/mL; $t = 1.444$; $df = 96$; $p = 0.153$). In contrast, post-treatment CRP was significantly lower in the combination group by 1.00 mg/L ($t = 6.208$; $df = 96$; $p < 0.001$), indicating a substantially greater anti-inflammatory effect on CRP with adjunctive nimesulide.

Table 4. Multiple Linear Regression Analysis Predicting IL-6 Levels Post-Treatment (IL-6_post)

Predictor Variable	B (Unstandardized)	SE	Beta (Standardized)	t	p-value
(Constant)	2.857	1.145	—	2.496	.014
LH_post	−0.063	0.045	−0.112	−1.415	.161
FSH_post	−0.031	0.039	−0.065	−0.789	.432
DHEAS_post	0.001	0.001	0.100	1.257	.212
TG_post	−0.002	0.003	−0.045	−0.580	.563
HDL_post	0.006	0.013	0.035	0.437	.663
Cholesterol_post	0.004	0.000	0.653	8.540	<.001
Free_Tes_post	0.109	0.041	0.205	2.641	.010
CRP_post	0.073	0.111	0.061	0.652	.516
Group	−0.407	0.223	−0.182	−1.819	.072

Footnote. Multiple linear regression with IL-6_post as the dependent variable; predictors as listed; two-tailed $\alpha = 0.05$ with significance indicated by $p < 0.05$.

Description. The multivariable model explained approximately half of the variance in post-treatment IL-6 ($R^2 = 0.516$; overall $p < .001$). Among the predictors, post-treatment total cholesterol showed a strong positive association with IL-6 ($B = 0.004$; $p < .001$), and post-treatment free testosterone was also positively associated ($B = 0.109$; $p = .010$). Other covariates, including LH_post, FSH_post, DHEAS_post, triglycerides, HDL, CRP_post, and treatment group, did not reach conventional statistical significance, although the group effect trended toward lower IL-6 with combination therapy ($B = -0.407$; $p = .072$). These findings indicate that lipid status and androgenic milieu are key correlates of residual inflammatory activity after treatment, while the adjusted group effect aligns directionally with the unadjusted comparisons observed elsewhere in the chapter.

B. Discussion

The current study evaluated the effects of metformin alone on inflammatory markers in women with PCOS and found significant reductions in both IL-6 and CRP over a short three-week treatment period. Specifically, IL-6 decreased from 5.70 ± 0.76 pg/mL to 4.00 ± 0.73 pg/mL and CRP from 7.24 ± 0.89 mg/L

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to 6.43 ± 0.73 mg/L, with highly significant p-values (<0.0001 for both). These findings suggest that even a relatively brief course of metformin can elicit a meaningful anti-inflammatory effect in women with PCOS. The marked t-values (21.79 for IL-6 and 14.79 for CRP) reinforce the robustness of this within-group change.

This observation aligns closely with a 2024 study by Al-Burhan et al., which also reported significant reductions in inflammatory markers, including high-sensitivity CRP, in women with PCOS after metformin treatment. However, the combination of metformin and omega-3 fatty acids showed even greater reductions, suggesting a synergistic anti-inflammatory benefit when metformin is paired with additional agents [19].

Likewise, a study by Kurbat and Swadi (2024) observed that the effects of using metformin with myoinositol in the management of hs-CRP and IL-18 were greater than those of metformin used alone, indicating again that they could be additive. These results align with the anti-inflammatory nature of metformin suggested by the present study, albeit that they postulate that combination treatments can be of better effect[20].

Additional support is provided by a 2022 randomized controlled trial by Elbandrawy et al., which compared metformin with aerobic exercise to yield better results with metformin alone. All the groups had a decrease in IL-6 and CRP with the combination group responding significantly more [21]. This is in line with the findings of the present study but once again implies that monotherapy might not necessarily be the most significant intervention.

Conversely, a meta-analysis published in 2017 by Wang et al. with 20 studies as subjects concluded that whereas CRP was significantly reduced under metformin therapy (SMD = -0.86), overall changes in IL-6 were statistically nonsignificant (SMD = -0.48, $p > .05$) [22]. This somewhat contradicts the current research data, specifically in the case of IL-6, and indicates the ad hoc heterogeneity of the anti-inflammatory actions of metformin in different populations and study designs.

With further details, a study by Víctor et al. also found that the IL-6 and other cytokines declined significantly with a 12-week period of metformin in patients with PCOS that is similar to the current study [23]. Nevertheless, it was also in this study where alterations in endothelial interactions were identified indicating that metformin had a wider systemic effect than the biomarkers measured.

On the other hand, anti-inflammatory effect of metformin is not always supported by evidence. Dardzi, et al. randomized crossover trial did not report statistically significant effects of four months of metformin therapy to reduce IL-6 or CRP and may indicate a potentially small, slow anti-inflammatory response in certain women with PCOS [24]. This deviation could be explained by duration of a study, the characters of the participants or level of baseline inflammation.

The variations in these results highlight the complicated interactions between the PCOS pathology, the course of treatment, the initial metabolic conditions, and the inflammatory reaction. The limited time, but great results of the present study suggest that in some of the populations, namely those with an elevated background inflammation or a more severe metabolic dysregulation, metformin might have fast acting

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effects. On the other hand, when there are inconsistent results when studies are longer, this could be a plateau effect or other lifestyle factors that could be causing variation in adherence or genetic factors.

The present study assessed the anti-inflammatory effect of a combination of metformin and nimesulide over a three-week intervention period in women with PCOS, as shown in Table 2. Notably, both interleukin-6 (IL-6) and C-reactive protein (CRP) levels significantly decreased following treatment. IL-6 dropped from 5.71 ± 1.00 pg/mL to 3.68 ± 1.40 pg/mL (mean difference 2.03 pg/mL, $p < 0.001$, Cohen's d = 1.703), while CRP declined from 7.20 ± 1.13 mg/L to 5.42 ± 0.87 mg/L (mean difference 1.78 mg/L, $p < 0.001$, Cohen's d = 1.898). These effect sizes are classified as very large, indicating a strong within-group anti-inflammatory response associated with the combination therapy.

Comparatively, several recent studies have investigated metformin's effect on inflammatory markers in women with PCOS, with mixed findings. A comprehensive meta-analysis by Wang et al. concluded that metformin significantly reduces serum CRP but does not significantly affect IL-6 levels in PCOS patients. This result partially supports the current study's findings, particularly regarding CRP, but diverges on IL-6 reduction, which was significant in the present study [22].

Adding further support, Al-Burhan et al. recently showed that combining metformin with omega-3 significantly reduced both hs-CRP and IL-18 more than metformin alone, reinforcing the concept that adjunctive anti-inflammatory agents potentiate metformin's effects—similar to the synergistic impact observed in the current study [19].

In contrast, a randomized trial by Xu et al. found that most included studies showed no significant changes in IL-6 levels after metformin treatment. Only one out of five studies demonstrated a reduction in IL-6, aligning with findings that metformin alone may have inconsistent effects on IL-6, which may help explain why the addition of nimesulide in the present study achieved stronger IL-6 reductions [25].

A study by Sathyapalan et al. offers further validation by showing that a combination of atorvastatin followed by metformin significantly decreased IL-6 and CRP levels in women with PCOS. This supports the broader idea that combination therapies, whether involving statins or NSAIDs like nimesulide, may be more effective in mitigating inflammatory responses than monotherapy with metformin alone [26].

However, the findings of Jakubowska et al. challenge the present study by reporting no significant changes in CRP or IL-6 after six months of metformin in obese PCOS women. This could be attributed to differences in baseline inflammation, BMI, treatment duration, or the absence of adjunctive therapy, all of which are potential modifiers of inflammatory outcomes [27].

The disparity between the present findings and some previous studies—especially regarding IL-6—may be attributed to the addition of nimesulide, a selective COX-2 inhibitor with known anti-inflammatory effects. While prior research has focused mainly on metformin, the use of nimesulide may offer an additional mechanism, such as inhibition of prostaglandin synthesis and TNF-α, leading to amplified reductions in IL-

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6 and CRP. This aligns with known COX-2 inhibition pathways and is consistent with findings from NSAID research, though nimesulide-specific data in PCOS is still limited.

In conclusion, the present study demonstrates that the addition of nimesulide to metformin produces substantial reductions in inflammatory markers IL-6 and CRP in women with PCOS, exceeding the anti-inflammatory efficacy reported for metformin alone in several previous studies. This suggests a promising role for adjunctive NSAID therapy in the management of chronic inflammation in PCOS, though further randomized controlled trials are warranted to confirm efficacy and safety across broader populations.

The present study evaluated the post-treatment differences in inflammatory biomarkers, IL-6 and CRP, between women with PCOS treated with metformin alone versus those treated with metformin plus nimesulide. The findings revealed that although both groups exhibited reductions in these markers, the difference in IL-6 levels between the two groups was not statistically significant ($p = 0.153$). However, a substantial and statistically significant reduction in CRP was observed in the combination group compared to the metformin-only group (mean difference = 1.00 mg/L; $p < 0.001$). This suggests that the addition of nimesulide—a COX-2 selective NSAID—might enhance the anti-inflammatory effect of metformin, particularly in reducing systemic CRP levels.

Comparatively, a 2022 randomized controlled trial by Elbandrawy et al. showed that the combination of aerobic exercise and metformin led to greater reductions in both IL-6 and CRP compared to metformin alone in PCOS patients, supporting the current study's finding that combination approaches yield stronger anti-inflammatory outcomes [21].

Additionally, a 2024 interventional study by Al-Burhan et al. reported that omega-3 supplementation alongside metformin significantly reduced both IL-18 and hs-CRP in PCOS patients, with greater reductions compared to metformin monotherapy. These findings again align with the present study's results on CRP, highlighting the potential of anti-inflammatory adjuncts with metformin [19].

On the other hand, in contrast to the current study's finding of a non-significant difference in IL-6 levels post-treatment, a 2021 meta-analysis by Karbalaee-Hasani et al. concluded that while CRP levels decreased significantly with metformin in type 2 diabetic patients, IL-6 reductions were not statistically significant, which aligns with the present study's outcome regarding IL-6 [28].

However, opposing evidence comes from a 2025 systematic review by Bansal et al., which found consistently elevated IL-6 levels in women with PCOS and suggested IL-6 as a reliable biomarker for systemic inflammation in this population. This supports the importance of detecting IL-6 changes and may argue for more sensitive or prolonged interventions to capture statistically significant differences [29].

Further divergence is seen in a 2024 rat model study by Coşar et al., which compared glutathione, metformin, and Diane-35, and found significant reductions in both IL-6 and CRP in the glutathione and metformin groups. This implies that metformin, even alone, may be effective in reducing IL-6 when appropriately dosed or studied in different models [30].

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Taken together, the present study's results are partially consistent with recent literature: the significant reduction in CRP with adjunctive therapy is robustly supported across several studies, while the IL-6 outcome remains less conclusive. This discrepancy could be due to the relatively short treatment duration (3 weeks), differences in assay sensitivity, or biological variability in IL-6 expression. The stronger CRP response may also reflect its downstream position in the inflammatory cascade, being more stable and sensitive to pharmacologic modulation than IL-6, which fluctuates more dynamically and may require longer interventions to show significant between-group changes.

The current study explored predictors of residual inflammation in women with PCOS following three weeks of treatment with either metformin alone or metformin plus nimesulide, using multiple linear regression to model post-treatment IL-6 levels. The model accounted for approximately 52% of the variance in IL-6 ($R^2 = 0.516$, $p < .001$), indicating a strong overall fit. Two biochemical predictors emerged as significant: total cholesterol ($B = 0.004$, $p < .001$) and free testosterone ($B = 0.109$, $p = .010$), both positively associated with IL-6. The treatment group variable showed a trend toward lower IL-6 with combination therapy ($B = -0.407$, $p = .072$), but this did not meet conventional significance. These findings highlight that even after controlling for treatment and metabolic factors, lipid and androgen profiles play a central role in sustaining inflammatory activity, underscoring the complexity of immune-endocrine interactions in PCOS.

The positive association between total cholesterol and IL-6 in the present study aligns with recent findings that emphasize the synergy between dyslipidemia and inflammation in cardiovascular and metabolic disease. In a large secondary analysis from the Cardiovascular Inflammation Reduction Trial, Ridker et al. found that both IL-6 and LDL-C independently predicted major cardiovascular events, and their combination dramatically increased risk. Their results underscore the persistent inflammatory risk associated with elevated cholesterol, even in aggressively treated patients [31].

Likewise, Gager et al. reported that IL-6 outperformed CRP as a predictor of long-term cardiovascular mortality in patients with acute coronary syndrome, reinforcing the clinical importance of IL-6 as a downstream marker of lipid-induced vascular inflammation [32].

In contrast, not all studies have found a consistent link between lipids and IL-6. For example, a population study by Fraser et al. concluded that after adjusting for confounders like smoking and lung function, IL-6's association with coronary events was no longer statistically significant, suggesting the role of IL-6 may vary by demographic and clinical context [33].

As for free testosterone, its positive association with IL-6 in the current study is consistent with prior evidence linking hyperandrogenism to pro-inflammatory states in PCOS. Walch et al. identified a polymorphism in the IL-6 promoter gene associated with higher androgen levels and metabolic dysregulation in women with PCOS, suggesting that genetic factors may mediate this relationship [34].

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Supporting this, Chaftari et al. demonstrated that IL-6 levels increase in non-febrile cancer patients with advanced disease and higher androgen exposure, reinforcing IL-6's role as a broader marker of chronic inflammation beyond infectious settings [35].

On the other hand, not all literature supports a robust androgen–IL-6 relationship. For example, in a COVID-19 pneumonia cohort, Patil et al. found that IL-6 was more influenced by disease severity and oxygen desaturation than hormonal or metabolic markers, suggesting that context- and pathology-specific mechanisms may mediate IL-6 elevation [36].

Lastly, the group effect in the current study—where IL-6 tended to be lower in the metformin plus nimesulide arm—adds clinical weight to the therapeutic potential of combining insulin sensitizers with anti-inflammatory agents in PCOS. While not statistically significant in the adjusted model, this trend corroborates findings from prior trials that show anti-inflammatory agents like NSAIDs can reduce systemic IL-6, especially in metabolically inflamed populations [32].

In conclusion, the present study confirms that total cholesterol and free testosterone are key independent predictors of post-treatment IL-6 levels in women with PCOS. These findings are in broad agreement with recent literature affirming the central roles of lipid dysregulation and androgen excess in sustaining systemic inflammation. Nonetheless, variations in context, comorbidity, and demographic characteristics across studies suggest that IL-6's predictive value may be modified by underlying pathophysiological mechanisms.

Conclusion

Adjunctive nimesulide with metformin in women with PCOS produced large within-group reductions in IL-6 and CRP over three weeks, with a significant post-treatment advantage versus metformin alone for CRP but not for IL-6. Multivariable modeling showed that higher total cholesterol and free testosterone independently predicted higher post-treatment IL-6, while the treatment group showed a non-significant trend toward lower IL-6 with combination therapy. These findings support targeting inflammation in PCOS through combined metabolic and anti-inflammatory strategies, and they highlight lipid and androgen profiles as meaningful correlates of residual inflammatory activity. Confirmation in rigorously randomized, longer-duration trials—incorporating standardized safety monitoring for NSAID co-therapy—is warranted.

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