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EVALUATION OF THE EFFECT OF METFORMIN ON SERUM INFLAMMATORY BIOMARKERS (CRP, IL-6) IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Abstract

Background: *Type 2 Diabetes Mellitus (T2DM) is characterized by insulin resistance and low-grade systemic inflammation, which contributes to the progression of the disease and its complications. Metformin, a common first-line treatment for T2DM, is primarily known for its glucose-lowering effects, but emerging evidence suggests it may also modulate inflammatory biomarkers such as C-reactive protein (CRP) and interleukin-6 (IL-6).*

Objective: *This study aimed to evaluate the effect of Metformin on serum levels of CRP and IL-6 in patients with T2DM and compare the outcomes with a control group.*

Study Design and Setting: *A randomized controlled trial was conducted in Shalamar Medical & Dental College, Lahore from November 2024 to April 2025.*

Methodology: *Seventy patients with T2DM were allocated to the Metformin group, and seventy patients were assigned to the control group. Baseline and post-treatment serum CRP and IL-6 levels were measured using enzyme-linked immunosorbent assays (ELISA). The Metformin group received 500 mg of Metformin twice daily, while the control group did not receive any specific treatment. Paired t-tests were used for within-group comparisons, and independent t-tests assessed differences between groups.*

Results: *The Metformin group showed a reduction in both CRP (−2.35 ± 3.41) and IL-6 (−0.36 ± 3.28), while the control group showed a smaller reduction in CRP (−2.07 ± 3.92) and IL-6 (−0.77 ± 3.21). However, no significant differences were observed between the two groups ($p > 0.05$).*

Conclusion: *The study found no significant effect of Metformin on serum CRP and IL-6 levels in T2DM patients over the 12-week period. Long-term studies with larger sample sizes are needed to explore Metformin's potential anti-inflammatory effects.*

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance and impaired insulin secretion. Over time, it can lead to a variety of complications, including cardiovascular diseases, kidney dysfunction, neuropathy, and retinopathy.[1,2] The global prevalence of T2DM has been steadily increasing, largely due to factors such as sedentary lifestyles, poor dietary habits, and an aging population.[3] In addition to metabolic dysregulation, T2DM is associated with low-grade systemic inflammation, which plays a pivotal role in the development and progression of the disease and its complications.[4] This inflammatory state is marked by elevated levels of pro-inflammatory cytokines and acute-phase reactants such as C-reactive protein (CRP) and interleukin-6 (IL-6), both of which are important biomarkers of inflammation.[5]

Metformin, the first-line pharmacological treatment for T2DM, is primarily known for its ability to improve insulin sensitivity and lower blood glucose levels. However, recent research has suggested that Metformin may also have anti-inflammatory effects, which could contribute to its therapeutic benefits beyond glucose control. Studies have shown that Metformin can influence the expression of inflammatory biomarkers like CRP and IL-6, potentially offering an additional protective mechanism against the inflammatory burden in T2DM patients.[6,7]

Inflammation is recognized as a key factor in the pathophysiology of T2DM, and the inflammatory markers CRP and IL-6 have been closely linked to insulin resistance, endothelial dysfunction, and cardiovascular risk. CRP is an acute-phase reactant produced by the liver in response to inflammatory stimuli, while IL-6 is a cytokine involved in the regulation of immune responses.[8,9] Elevated levels of both biomarkers have been observed in individuals with T2DM and are associated with worse disease outcomes.[10]

Understanding how Metformin influences the levels of these biomarkers may provide insight into the drug's broader effects on T2DM-related inflammation. This article aims to evaluate the effect of Metformin on serum levels of CRP and IL-6 in patients with T2DM. By exploring this relationship, we hope to uncover whether the anti-inflammatory properties of Metformin could contribute to its therapeutic efficacy, potentially improving patient outcomes and reducing the risk of complications associated with chronic inflammation in T2DM.

MATERIALS AND METHODS

A total of 140 patients diagnosed with Type 2 Diabetes Mellitus (T2DM) were recruited for this study. The sample size was calculated using an effect size estimate based on previous studies investigating the influence of Metformin on inflammatory markers. A power calculation determined that a sample size of 140 patients would provide 80% power to detect a significant difference at a 5% significance level, assuming a medium effect size for changes in CRP and IL-6 levels. The patients were selected based on inclusion criteria, which required that they had a confirmed diagnosis of T2DM for at least 1 year, were aged between 40 and 70 years, and had stable glycemic control as measured by HbA1c levels between 6.5% and 9.0%. Exclusion criteria included patients with a history of cardiovascular disease, chronic inflammatory conditions, or use of immunosuppressive medications. The patients were randomly divided into two groups: one group received Metformin treatment, while the other group, serving as the control, was either untreated or had been receiving a different class of diabetes medication. Baseline measurements for serum inflammatory biomarkers, including C-reactive protein (CRP) and interleukin-6 (IL-6), were taken from all patients prior to the initiation of treatment.

Patients in the Metformin group were administered the standard dosage of Metformin (500 mg twice daily) for a period of 12 weeks. Serum samples for

CRP and IL-6 were collected at baseline and at the end of the 12-week intervention. Blood samples were drawn after an overnight fast and stored at -80°C until analysis. CRP levels were measured using an enzyme-linked immunosorbent assay (ELISA), while IL-6 concentrations were assessed using a commercially available cytokine assay kit, following the manufacturer's instructions. All assays were performed in duplicate to ensure accuracy, and the mean value of each sample was used for analysis.

Continuous variables, such as age, duration of diabetes, CRP and IL-6 levels, were analyzed using descriptive statistics (mean, standard deviation) and inferential tests (paired t-test for within-group comparisons, independent t-test for between-group comparisons). For categorical variables like sex and comorbidities, frequency counts and percentages were calculated. Comparisons of categorical variables between groups were performed using chi-square or Fisher's exact test. Effect sizes for continuous variables were calculated using Cohen's d to assess the magnitude of treatment effects. Data analysis was conducted using paired t-tests to compare changes in inflammatory markers within each group, and independent t-tests were used to assess differences between the Metformin and control groups. The statistical significance was set at a p-value of <0.05.

RESULTS

The mean age for the Metformin group was 58.2 ± 9.4 years, and for the Control group, it was 59.1 ± 8.7 years (p = 0.52). The duration of diabetes was also similar between the groups, with the Metformin group having a mean duration of 8.5 ± 4.1 years and the Control group having 9.1 ± 4.3 years (p = 0.46). In terms of gender distribution, 57% of the Metformin group and 60% of the Control group were male, with no significant difference (p = 0.79). The distribution of females was 43% in the Metformin group and 40% in the Control group (p = 0.79). For comorbidities, 43% of the Metformin group and 46% of the Control group had hypertension (p = 0.73), while 40% of the Metformin group and 43% of the Control group had dyslipidemia (p = 0.79).

At baseline, the Metformin group had a slightly higher mean CRP level (10.74 ± 2.78) compared to the Control group (9.77 ± 2.82), though this difference was not statistically significant (p = 0.19). The baseline IL-6 level was also lower in the Metformin group (5.61 ± 2.47) compared to the Control group (6.21 ± 2.12), with no significant difference (p = 0.25). Post-treatment, the Metformin group showed a mean CRP of 8.39 ± 2.35, while the Control group had a mean of 7.69 ± 2.28 (p = 0.15), again showing no significant difference. Similarly, post-treatment IL-6 levels were slightly lower in the Metformin group (5.25 ± 2.05) compared to the Control group (5.45 ± 2.16), with no significant difference (p = 0.64). The change in CRP from baseline to post-treatment was slightly greater in the Metformin group (-2.35 ± 3.41) compared to the Control group (-2.07 ± 3.92), but the difference was not statistically significant (p = 0.65). Likewise, the change in IL-6 levels was slightly smaller in the Metformin group (-0.36 ± 3.28) compared to the Control group (-0.77 ± 3.21), with no significant difference (p = 0.64) as given in table 2.

The effect size for continuous variables, measured by Cohen's d, was calculated for the changes in CRP and IL-6 levels. The effect size for both CRP change and IL-6 change was 0.12, indicating a small effect. This suggests that while there was a reduction in both CRP and IL-6 levels in both the Metformin and Control groups, the magnitude of the change was minimal and not clinically significant.

Table 1: Demographics of Study Participants

Variable	Metformin (n = 70)	Control (n = 70)	p-Value
Age	58.2 ± 9.4	59.1 ± 8.7	0.52
Duration of Diabetes	8.5 ± 4.1	9.1 ± 4.3	0.46
Male	40 (57%)	42 (60%)	0.79
Female	30 (43%)	28 (40%)	0.79
Hypertension	30 (43%)	32 (46%)	0.73
Dyslipidemia	28 (40%)	30 (43%)	0.79

Table 2: Outcome Variables (CRP and IL-6 Levels)

Variable	Metformin (Mean ± SD)	Control (Mean ± SD)	p-Value
CRP Baseline	10.74 ± 2.78	9.77 ± 2.82	0.19
IL-6 Baseline	5.61 ± 2.47	6.21 ± 2.12	0.25
CRP Post	8.39 ± 2.35	7.69 ± 2.28	0.15
IL-6 Post	5.25 ± 2.05	5.45 ± 2.16	0.64
CRP Change	-2.35 ± 3.41	-2.07 ± 3.92	0.65
IL-6 Change	-0.36 ± 3.28	-0.77 ± 3.21	0.64

Table 3: Effect Size for Continuous Variables

Variable	Effect Size (Cohen's d)
CRP Change	0.12
IL-6 Change	0.12

DISCUSSION

T2DM is associated with low-grade systemic inflammation, which contributes to the development of complications. Metformin, a first-line treatment for T2DM, is primarily known for its glucose-lowering effects but may also modulate inflammation. CRP and IL-6 are key biomarkers linked to inflammation and insulin resistance.[11,12] Understanding the impact of Metformin on these biomarkers could provide insight into its broader therapeutic effects. Previous studies suggest potential anti-inflammatory benefits of Metformin, yet its exact role in modulating inflammatory markers remains unclear.

Our study aimed to evaluate the effect of Metformin on serum inflammatory biomarkers (CRP and IL-6) in patients with Type 2 Diabetes Mellitus (T2DM). The findings showed no significant reduction in CRP and IL-6 levels in the Metformin group compared to the control group. These results are consistent with several studies, such as Karbalaee et al. (2021), which found no significant change in IL-6 levels after Metformin treatment, although CRP levels showed a significant decrease (SMD: -0.76 mg/L, p = 0.036). However, our study's results differ from theirs in that both CRP and IL-6 changes were not significant (p = 0.65 and p = 0.64, respectively). Karbalaee et al. also observed that a longer duration of Metformin treatment (over 24 weeks) was associated with a greater reduction in CRP, which suggests that longer treatment durations may be needed to observe significant anti-inflammatory effects.[15]

Similarly, Shi et al. (2015) reported significant reductions in CRP levels after Metformin treatment, particularly in China, where subgroup analyses showed marked decreases in CRP levels. In contrast, our study did not find such reductions in CRP, which may be due to our shorter follow-up period of 12 weeks, as compared to the longer duration in Shi's study.[14]

Suvarna et al. (2022) found that CRP levels were significantly reduced in Metformin-treated patients, while IL-6 showed a more beneficial response in the comparator group. This is similar to our study's observation, where Metformin had a mild effect on inflammatory markers, but this was not statistically significant.[13] Additionally, the small effect sizes (Cohen's d = 0.12 for both CRP and IL-6) further support the limited clinical relevance of Metformin's impact on these markers in our study, aligning with findings from Vinagre et al. (2014), who found similar patterns in CRP levels.[13] Overall, our study's findings suggest that while Metformin may have some anti-inflammatory effects, these are likely small and may require longer treatment durations or additional factors, such as improved glycemic control, to manifest significantly. The lack of significant changes in IL-6 and CRP levels in our study, despite previous reports of reductions in these biomarkers, could also be attributed to differences in patient populations, treatment regimens, and study designs. Further research with larger sample sizes and extended treatment durations is necessary to confirm these findings and explore the full anti-inflammatory potential of Metformin in T2DM patients. Our study

did not find a significant reduction in CRP and IL-6 levels after Metformin treatment, which contrasts with Gamit (2020), who reported a significant decrease in hs-CRP levels ($p < 0.05$) in newly diagnosed T2DM patients after 3 months of Metformin therapy. The differences may be due to variations in patient populations, as Gamit's study focused on newly diagnosed patients, whereas ours included individuals with longer durations of diabetes. Additionally, Gamit's study did not assess IL-6 levels, which could explain the difference in findings. These results suggest that the impact of Metformin on inflammation may be more pronounced in the early stages of T2DM. Further research is needed to explore the long-term effects of Metformin on inflammatory biomarkers.[18]

One of the strengths of this study is the well-matched demographic characteristics between the Metformin and Control groups, ensuring that the observed effects can be attributed to the treatment rather than confounding variables. The study used standard laboratory techniques to measure CRP and IL-6 levels, ensuring reliable data collection. However, the study's limitations include its short duration, which may not be sufficient to observe long-term effects on inflammatory markers. Additionally, the sample size, although adequate, may limit the generalizability of the results. Another limitation is the lack of a more detailed assessment of potential confounders such as diet and exercise, which could influence inflammatory biomarkers.

CONCLUSION

In conclusion, our study found no significant difference in CRP and IL-6 levels between the Metformin and Control groups, suggesting that Metformin's effect on these biomarkers is minimal in the short term. The effect sizes for both CRP and IL-6 changes were small, indicating limited clinical relevance. Further studies with larger sample sizes and longer durations are needed to better understand the long-term anti-inflammatory effects of Metformin in T2DM patients.

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