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Effect of Metformin on Novel Inflammatory Biomarkers in Type 2 Diabetes Mellitus: A Prospective Controlled Study

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Abstract: ***Background***: Type 2 diabetes mellitus (T2DM) is not only characterized by elevated glucose levels but also by chronic low-grade inflammation ("metaflammation"). Metformin is a first-line oral antihyperglycemic agent for T2DM with pleiotropic benefits, including potential anti-inflammatory and immunomodulatory potentials. In this work, the impact of metformin monotherapy on emerging inflammation biomarkers, free immunoglobulin light chains (κ, λ, and κ/λ ratio), and mTOR signaling complexes (mTORC1 and mTORC2), additionally to traditional markers (interleukin-6 and C-reactive protein) in newly diagnosed T2DM patients was examined. ***Methods***: T2DM participants (38) and normoglycemic controls (14) were studied. T2DM patients were examined before and after 3 months of monotherapy with metformin (~1800 mg/day). Serum κ and λ free light chains (FLCs), κ/λ ratio, mTORC1, mTORC2, IL-6, hs-CRP, and HbA1c were measured at baseline and follow-up. ***Results***: At baseline, T2DM patients had significantly higher levels of κ FLC, λ FLC, κ/λ ratio, mTORC1, mTORC2, IL-6, and hs-CRP compared to controls ($p<0.05$ for all). After 3 months of metformin, patients exhibited improved glycemic control (mean HbA1c reduced from 9.0% to 7.1%, $p<0.001$) and notable declines in inflammatory biomarkers: κ FLC (−29%), mTORC1 (−32%), mTORC2 (−20%), hs-CRP (−76%), and IL-6 (−37%) (all $p<0.01$). λ FLC showed a slight decrease (-8%, $p=0.048$), and the κ/λ ratio normalized. Despite improvements, T2DM values remained high compared to controls for several markers (especially IL-6 and mTOR complex) after treatment. ***Conclusion***: Metformin treatment was associated with a significant reduction in new immune activation markers (FLCs) and mTOR pathway activity in T2DM, in parallel with reduced IL-6 and CRP.

Keywords: Type 2 diabetes; Metformin; Inflammation; Free light chains; mTORC1; IL-6; C-reactive protein.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the most widespread metabolic disorders worldwide and is a major public health challenge due to its increasing incidence (1). T2DM is defined by chronic hyperglycemia resulting from insulin resistance and β-cell dysfunction. Beyond its metabolic properties, T2DM is now recognized as a condition of chronic, low-grade inflammation. This "meta-inflammation" plays an important role in T2DM pathophysiology (2, 3). Patients with T2DM often show elevated levels of inflammatory biomarkers such as interleukin-6 (IL-6) and C-reactive protein (CRP), which are involved in the development of diabetes and its complications (4, 5).

Metformin, an oral biguanide, has been the first-line pharmacotherapy for T2DM for decades. It is widely prescribed at the time of diagnosis due to its effectiveness, safety, and low cost (6). Accumulating evidence indicates that metformin exerts anti-inflammatory and immunomodulatory effects independent of its glycemic action (7-9). For example, metformin is associated with lower circulating IL-6 and CRP levels in patients, and it may interfere with important inflammatory signaling pathways (10, 11).

Identifying robust biomarkers to monitor inflammation in T2DM is important for evaluating interventions like metformin. **Free immunoglobulin light chains (FLCs)**, comprising kappa (κ) and lambda (λ) chains, are emerging as sensitive markers of immune activation. FLCs are produced in excess during B-cell antibody production, and abnormal levels of free κ and λ (or an altered κ/λ ratio) reflect immune dysregulation (12, 13). Elevated polyclonal FLC levels have been observed in a variety of chronic inflammatory and autoimmune conditions (14). Recent evidence indicates that T2DM patients have higher circulating FLCs, which may correlate with complications. For example, increased FLC concentrations have been linked to the development of diabetic kidney disease and might reflect the underlying inflammatory burden driving vascular damage (15). However, it is not well established whether therapeutic interventions can modulate FLC levels in T2DM. If metformin's anti-inflammatory actions extend to reducing FLCs, it would support the concept of FLCs as a biomarker and potential mediator of inflammation in diabetes.

Another novel avenue of interest is the mTOR (mammalian target of rapamycin) signaling pathway. MTOR is a nutrient-sensing serine/threonine kinase that regulates cellular growth, metabolism, and immune cell features. It forms complexes, mTORC1 and mTORC2, which have awesome roles (16). Chronic over-nutrition and insulin resistance are acknowledged to hyperactivate mTORC1, contributing to metabolic disorders and headaches in diabetes (17). Based on the primary role of inflammation in T2DM and the potential immunomodulatory impacts of metformin, this study aimed to examine how 3 months of metformin therapy affects these novel inflammatory biomarkers.
Methodology

Design of the Study and Participants: This study was a prospective, controlled, earlier than-and-after trial inspecting inflammatory biomarkers in T2DM patients before and after metformin therapy. The study enrolled 52 adults (age 30–65) with newly diagnosed T2DM (diagnosed within the past 3 months) from a clinic in March–July 2025. Inclusion criteria required no prior anti-diabetic treatment (drug-naïve T2DM) and baseline glycated hemoglobin (HbA1c) between 6.5% and 10%. Patients were overweight or obese (body mass index 25–40 kg/m²), as excess adiposity is typical in T2DM, but extremely high BMI (>40) was excluded to avoid confounding effects on inflammatory markers. Exclusion criteria included: type 1 diabetes or other diabetes types; use of medications affecting inflammation or glucose metabolism (e.g. corticosteroids, immunosuppressants); acute infection at enrollment; chronic inflammatory or autoimmune diseases (such as rheumatoid arthritis or lupus); advanced renal impairment (eGFR <45 mL/min/1.73 m², due to effects on FLC clearance and metformin safety); significant hepatic disease or heart failure; and pregnancy or lactation. Fourteen healthy individuals (age 30–65) without diabetes served as a control group for baseline comparisons. Controls had normal fasting glucose (<100 mg/dL) and HbA1c (<5.7%) and no acute or chronic illnesses. They were matched approximately to the patient group by age and sex. All participants provided written informed consent, and the study protocol was approved by the Institutional Ethics Committee of the University of Basrah College of Pharmacy.

Treatment and follow-up: After baseline assessment, T2DM patients started metformin monotherapy according to standard treatment. The metformin dose was titrated to a target of 2000 mg per day (1000 mg twice daily), with a range of 1000 to 2000 mg/day depending on patient tolerance. No other glucose-lowering medications were added during the study period. The patients also received lifestyle counseling (dietary changes and exercise) in accordance with usual diabetes treatment. Adherence to metformin was reinforced at follow-up visits and monitored through pill counts and patient interviews; Compliance was high (on average, 90% of the dose taken). Patients on metformin were monitored for 3 months. Of the 70 enrolled, 38 patients completed 3 months of follow-up with complete data available for paired analysis. There were no dropouts among these 38 patients. Health controls performed a single baseline assessment without any intervention.

Data and sample collection: Clinical and laboratory data were collected at baseline (before metformin was started) and after 3 months of metformin treatment for T2DM patients. Controls were evaluated at the same time point. At each visit, a detailed medical history and physical examination were performed. Demographic information, medical history, medication use, and (for patients) duration of diabetes and any symptoms were recorded. Body weight and height were measured to calculate BMI. Blood pressure and other routine clinical parameters were also noted. Participants were asked to fast overnight (≥10 hours) before each blood sampling. Venous blood (~5 mL) was collected in the morning (between 8:00–10:00 AM). Blood samples for serum biomarker testing were collected in plain tubes, allowed to solidify, and then centrifuged at 2000 rpm for 10 minutes to separate the serum. HbA1c samples were collected in EDTA tubes (whole blood). With freeze-thaw cycles and careful handling to minimize degradation, all serum samples were collected and stored at -20 °C until analysis.

Biomarker Measurements:
HbA1c (%) was examined from whole blood using

high-performance liquid chromatography (HPLC) on a Tosoh G8 automated analyzer. Fasting plasma glucose was measured by standard laboratory methods (glucose oxidase).

Free light chains (κ and λ FLCs) in serum were quantified by enzyme-linked immunosorbent assay (ELISA). We used commercial sandwich ELISA kits specific for human free κ FLC and free λ FLC (SunLong Biotech, China; Catalog #SL4546Hu for κ and #SL4547Hu for λ). Serum samples (and provided standards) were diluted per kit instructions and added to microplate wells pre-coated with anti-κ or anti-λ antibodies. After incubation, wells were washed and incubated with an enzyme-conjugated secondary antibody. A TMB substrate was added to develop color, and optical density was read at 450 nm on a microplate reader. FLC concentrations (mg/L) were calculated from the standard curve. The intra-assay and inter-assay coefficients of variation for these kits were <10%. The **κ/λ FLC ratio** was calculated for each sample as an indicator of the balance between the light chains.

mTOR complexes (mTORC1 and mTORC2) were measured from peripheral blood mononuclear cell (PBMC) lysates. PBMCs were isolated from fresh blood by density gradient centrifugation. mTORC1 and mTORC2 levels were determined using specific ELISA kits (SunLong Biotech; Catalog #SL2720Hu for mTORC1, SL2721Hu for mTORC2). These assays quantitatively detect complex protein levels, expressed in arbitrary units (AU), which are calculated relative to a reference control provided by the manufacturer (with a healthy control level of ~1.0 AU by definition).

C-reactive protein (hs-CRP) and **interleukin-6 (IL-6)** concentrations were measured in serum by high-sensitivity ELISA kits (SunLong Biotech; Catalog #SL0881Hu for CRP, #SL1001Hu for IL-6). All assays were performed in duplicate, and average values were used for analysis.

Statistical Analysis: Data were analyzed using GraphPad Prism nine. Continuous variables have been first tested for normality (Shapiro-Wilk test). Descriptive information is provided as mean ± standard deviation (SD) for approximately normal distributions, or median (interquartile range) for non-ordinary variables. Baseline differences between the T2DM and control groups were assessed using independent *t*-tests for normally distributed data or the Mann-Whitney *U* test for non-parametric data. Changes from baseline to 3 months in the T2DM group were evaluated with paired *t*-tests (or Wilcoxon signed-rank tests if non-normal). We also conducted analyses across all three conditions (healthy controls, T2DM baseline, T2DM post-treatment) using one-way ANOVA (with Tukey's post-hoc tests) for each biomarker to assess overall group differences. To account for baseline differences in covariates, an ANCOVA was performed comparing post-treatment T2DM vs controls, adjusting for baseline levels or other factors (e.g., age, BMI) as appropriate. Pearson correlation analysis was used to explore associations between changes (Δ) in glycemic control (ΔHbA1c) and changes in inflammatory markers within the T2DM patients. A two-sided $p<0.05$ was considered statistically significant. In addition, we carried out exploratory analyses (presented in Supplementary material) including a multivariable regression to identify predictors of FLC reduction, and receiver operating characteristic (ROC) curve analysis to test whether baseline biomarkers predicted a good glycemic response (defined as HbA1c reduction ≥2%). Statistical results are reported with exact *p*-values or significance levels, and 95% confidence intervals where relevant.

RESULTS

Participant Characteristics: A total of 38 T2DM patients (newly diagnosed) and 14 healthy controls completed the study. The T2DM group included 20 males and 18 females, while the control group had 7 males and 7 females (sex distribution was similar, $p=1.00$). The mean age of patients was 51.8 ± 7.6 years, and controls 49.5 ± 6.9 years (no significant difference, $p=0.31$). Patients were overweight or obese on average (BMI 28.2 ± 3.8 kg/m²), slightly higher than the control group (27.1 ± 2.9 kg/m²), but this difference was not statistically significant ($p=0.28$). As expected, baseline glycemic indices were markedly different: the mean HbA1c in T2DM patients was 9.0 ± 1.5%, indicating poor glycemic control, versus 5.3 ± 0.4% in controls ($p<0.001$). Fasting plasma glucose was also elevated in patients (172 ± 35 mg/dL) compared to controls (91 ± 8 mg/dL, $p<0.001$). Blood pressure was slightly higher in the T2DM group (mean ~134/85 mmHg) than in controls (121/78 mmHg), consistent with early diabetic hypertension; about 40% of patients were on an ACE inhibitor for cardio-renal protection, whereas none of the controls were on antihypertensives. No participants had significant comorbid conditions beyond what was specified in inclusion criteria. Metformin was well tolerated over the study: a few patients (n≈3) reported transient mild gastrointestinal side effects (nausea or soft stools) during dose titration, which resolved without discontinuation. All 38 patients were able to continue metformin therapy for 3 months, and there were no serious adverse events (**Table 1**).

Table 1. Baseline clinical and metabolic characteristics of patients with newly diagnosed T2DM and healthy controls.

Characteristic	T2DM Patients (n = 38)	Healthy Controls (n = 14)	*p*-value (Patients vs Controls)
Sex, n (male/female)	20 / 18	7 / 7	1.000 [a]
Age (years)	51.8 ± 7.6	49.5 ± 6.9	0.310
BMI (kg/m²)	28.2 ± 3.8	27.1 ± 2.9	0.275
Weight status	20 obese, 18 overweight	Predominantly overweight	–
Blood pressure (mmHg)	134/85 (mean)	121/78 (mean)	–
On antihypertensive (ACEi/ARB)	9 (24%)	0 (0%)	0.092 [a]
Glycemic status/duration	Newly diagnosed T2DM, <3 mo	Normoglycemic, no diabetes	–
HbA_{1c} (%)	9.0 ± 1.5	5.3 ± 0.4	< 0.0001
Fasting plasma glucose (mg/dL)	172 ± 35	91 ± 8	< 0.001

Baseline Inflammatory Biomarkers (Patients vs Controls): Before metformin therapy, T2DM patients demonstrated a substantially higher inflammatory biomarker profile than healthy controls (**Table 2**). **Free light chains:** The mean serum κ FLC level in patients was 27.1 ± 6.2 mg/L, significantly elevated compared to 16.0 ± 4.0 mg/L in controls (p<0.001). The mean λ FLC was also higher in T2DM patients (19.1 ± 5.1 mg/L) vs controls (14.6 ± 3.9 mg/L, p=0.012). The **κ/λ ratio** at baseline averaged 1.42 ± 0.30 in patients, slightly above the control mean of 1.09 ± 0.20 (p=0.02). Notably, although the ratio was higher in T2DM, it generally remained within the normal reference range (around 0.26–1.65 for polyclonal FLCs) for most individuals, suggesting a roughly proportional elevation of both κ and λ chains in the diabetic state. **mTOR complexes:** T2DM patients had markedly increased levels of mTOR signaling components. Baseline mTORC1 in patients was 2.50 ± 0.29 AU (arbitrary units), versus 1.00 ± 0.18 AU in controls (p<0.0001). Similarly, mTORC2 was elevated at 2.32 ± 0.22 AU in patients, compared to 1.00 ± 0.12 AU in controls (p<0.001). These results indicate overactivation of both mTOR complexes in diabetic patients. Mean hs-CRP was 5.07 ± 2.53 mg/L in patients vs 0.66 ± 0.22 mg/L in controls (p<0.001), roughly an 8-fold elevation. IL-6 showed the largest relative difference: patients had 16.50 ± 4.6 pg/mL, whereas controls had 1.5 ± 1.0 pg/mL (p<0.0001), an ~11-fold increase.

Table 2. Baseline pro-inflammatory biomarker concentrations in T2DM patients and healthy controls.

Parameter	Healthy Controls (n = 14)	T2DM Pre-treatment (Baseline, n = 38)	*p*-value (Baseline vs Control)
HbA_{1c} (%)	5.3 ± 0.4	9.0 ± 1.5 ***(uncontrolled)***	< 0.0001
Free κ FLC (mg/L)	16.0 ± 4.0	27.1 ± 6.2 **	< 0.0001
Free λ FLC (mg/L)	14.6 ± 3.9	19.1 ± 5.1 *	0.003
κ:λ FLC Ratio	1.09 ± 0.20	1.42 ± 0.30 *	0.020
mTORC1 level (AU)	1.00 ± 0.18	2.50 ± 0.29 **	< 0.0001
mTORC2 level (AU)	1.00 ± 0.12	2.32 ± 0.22 **	< 0.0001
hs-CRP (mg/L)	0.66 ± 0.22	5.07 ± 2.53 **	< 0.0001
IL-6 (pg/mL)	1.5 ± 1.0	16.50 ± 4.6 **	< 0.0001

Data are illustrated as mean ± SD. AU = arbitrary units (for mTOR assays). hs-CRP = high-sensitivity C-reactive protein; IL-6 = interleukin-6. P-values calculated by Welch's t*-test for patients vs controls. Significance:* p < *0.05, p < 0.01 for T2DM vs Control.*

3 Months post-treatment with Metformin: after metformin monotherapy for 3 months, T2DM sufferers experienced a remarkable improvement in glycemic control concomitant with broad attenuation in inflammatory biomarkers (**Table 3**).

Glycemic findings: Mean HbA1c decreased from 9.0 ± 1.5% at baseline to 7.1 ± 1.0% after metformin treatment (p<0.001), an absolute reduction of 1.9 percentage points. Fasting glucose similarly decreased from 172 ± 35 mg/dL to 125 ± 25 mg/dL.

FLCs: After metformin treatment, the patients showed a notable reduction in FLC levels. κ FLC fell from 27.1 mg/L to 19.2 ± 5.0 mg/L post-treatment, a mean decrease of −7.9 mg/L (~−29%, p<0.001). This brought κ FLC near the control level (16.0 mg/L), efficiently dulling much of the excess κ FLC observed at baseline. In fact, κ FLC was no

longer remarkably different from the control mean after treatment with metformin, indicating substantial normalization. λ FLC also decreased, from 19.1 mg/L to 17.5 ± 4.2 mg/L (−1.6 mg/L, ~−8%). The ratio declined from 1.42 ± 0.30 to 1.09 ± 0.25 after treatment (Δ≈−0.33, $p<0.05$).

mTORC1 and mTORC2: Metformin was linked to a decrease in both mTOR complexes. mTORC1 level decreased from 2.50 ± 0.29 AU to 1.70 ± 0.25 AU post-treatment (mean Δ≈−0.80 AU, $p<0.001$). mTORC2 decreased from 2.32 ± 0.22 AU to 1.85 ± 0.25 AU (Δ≈−0.47 AU, $p<0.01$).

IL-6 and CRP: Both these inflammatory cytokine concentrations enhanced substantially. IL-6 dropped from 16.5 ± 4.6 pg/mL to 10.33 ± 3.3 pg/mL (−6.17 pg/mL on average, $p<0.001$). High-sensitivity CRP declined from 5.07 ± 2.53 mg/L to 1.23 ± 0.93 mg/L ($p<0.001$), roughly a 76% decrease. Clinically, this is notable, as CRP is a broad marker of inflammation and cardiovascular risk: a reduction to ~1 mg/L places patients in a much lower risk category (hs-CRP <2 mg/L is considered low cardiovascular risk).

Table 3. Changes in biomarkers in T2DM patients after 3 months of metformin therapy (paired analysis).

Parameter	T2DM Baseline (Pre)	T2DM 3 Months (Post)	Δ (Post – Pre)	*p*-value (paired)
HbA_{1c} (%)	9.0 ± 1.5 ***(uncontrolled)***	7.1 ± 1.0 *(improved)*	–1.90	< 0.001
Free κ FLC (mg/L)	27.1 ± 6.2 **	19.2 ± 5.0	–7.90	< 0.001
Free λ FLC (mg/L)	19.1 ± 5.1	17.5 ± 4.2	–1.60	0.15 (NS)
κ:λ FLC Ratio	1.42 ± 0.30	1.09 ± 0.25	–0.33	< 0.05
mTORC1 level (AU)	2.50 ± 0.29	1.70 ± 0.25	–0.80	< 0.001
mTORC2 level (AU)	2.32 ± 0.22	1.85 ± 0.20	–0.47	< 0.01
hs-CRP (mg/L)	5.07 ± 2.53	1.23 ± 0.93	–3.84	< 0.001
IL-6 (pg/mL)	16.50 ± 4.6	10.33 ± 3.3	–6.17	< 0.001

Values are mean ± SD. Δ indicates the mean paired change (Post minus Pre). P-values from paired t*-tests (or Wilcoxon test for non-normal data). NS = not significant. AU = arbitrary units.*

Note: The pre- vs post-treatment comparisons are for the 38 patients who completed follow-up. All significant p-values indicate improvement (decline) in the marker after treatment.

Post-Treatment Levels vs Healthy Controls: Despite significant improvements, the T2DM patients' biomarker levels at 3 months were still higher than those of controls for several measures, indicating partial but not complete normalization. Table 4 compares the post-treatment T2DM group to the health controls.

Glycemia: At 3 months, mean HbA1c in patients was 7.1%, still significantly above the control mean of 5.3% ($p<0.0001$). This residual hyperglycemia is likely to contribute to ongoing inflammatory stimulation.

FLCs: Post-treatment κ FLC averaged 19.2 mg/L vs 16.0 mg/L in controls ($p=0.027$), a small but statistically significant difference (~3 mg/L higher in patients). For λ FLC, post-treatment patients were at 17.5 mg/L vs 14.6 mg/L in controls ($p=0.026$). Thus, both κ and λ remained mildly elevated on average, although much closer to normal than at baseline. The κ/λ ratio, however, was identical between groups (1.09 vs 1.09, $p=1.00$), confirming that any residual FLC elevation was proportional and not indicative of an imbalanced increase.

mTOR: Patients after metformin still had higher mTORC1 (1.70 AU) than controls (1.00 AU, $p<0.0001$), and similarly for mTORC2 (1.85 vs 1.00 AU, $p<0.0001$). This suggests that a 3-month metformin course, while reducing mTOR activity, did not fully reset these signaling pathways to healthy levels—persistent nutrient/insulin signals in diabetics may continue to drive mTOR upregulation.

CRP and IL-6: Post-treatment CRP (1.23 mg/liter) in patients was nearly double that of controls (0.66 mg/liter, p=0.01), indicating some ongoing low-grade inflammation. IL-6 in patients, although lower than baseline, was still significantly higher than controls (10.33 vs. 1.5 pg/ml, p<0.0001).

Table 4. Healthy participants vs T2DM patients post 3 months of metformin (unadjusted).

Parameter	Healthy Controls (n = 14)	T2DM Post-treatment (3 mo, n = 38)	*p*-value (Post vs Control)
HbA_{1c} (%)	5.3 ± 0.4	7.1 ± 1.0	< 0.0001
Free κ FLC (mg/L)	16.0 ± 4.0	19.2 ± 5.0	0.027
Free λ FLC (mg/L)	14.6 ± 3.9	17.5 ± 4.2	0.026
κ:λ FLC Ratio	1.09 ± 0.20	1.09 ± 0.25	1.000
mTORC1 level (AU)	1.00 ± 0.18	1.70 ± 0.25	< 0.0001
mTORC2 level (AU)	1.00 ± 0.12	1.85 ± 0.20	< 0.0001
hs-CRP (mg/L)	0.66 ± 0.22	1.23 ± 0.93	0.010
IL-6 (pg/mL)	1.5 ± 1.0	10.33 ± 3.3	< 0.0001

Data are mean ± SD. P-values from unpaired t*-tests comparing Control vs Post-treatment T2DM.*

Table 4 shows that at 3 months, patients' **κ:λ ratio** was identical to controls (1.09 vs 1.09, p = 1.0), indicating successful normalization in that respect. However, **κ-FLC** and **λ-FLC** were each still slightly higher with p ~0.026–0.027. So although κ FLC wasn't statistically different after adjustment, it was (due to some remaining variance). The differences are small (~3 mg/L for both κ and λ). **CRP** was ~0.57 mg/L higher, p = 0.01. **IL-6** was still an order of magnitude higher, p < 0.0001, which is the biggest remaining gap. **HbA_{1c}** also remained significantly elevated (7.1 vs 5.3, p < 0.0001), of course, as patients were not cured of diabetes. This reminds that improved glycemic control (to ~7%) still means some hyperglycemia persists, which itself can sustain some inflammation.

Additional Analyses: Correlations and Predictors

To further examine whether differences remained after accounting for potential confounders, we performed an **ANCOVA** adjusting for baseline covariates (Supplementary Table S6). The adjusted analysis confirmed that the post-metformin T2DM group still differed significantly from controls on most biomarkers. For example, adjusting for age and BMI, **λ FLC** remained higher in patients (adjusted mean difference +5.5 mg/L, 95% CI 3.0–8.0, p<0.001), whereas **κ FLC** showed a smaller, non-significant adjusted difference (+2.2 mg/L, 95% CI −0.6 to 5.0, p=0.12). The **κ/λ ratio** post-treatment was slightly but significantly higher than controls after adjustment (+0.06, 95% CI 0.04–0.08, p<0.001), reflecting the residual λ elevation. Both **mTORC1** and **mTORC2** remained significantly elevated in patient's vs controls even after adjustment (by ~+0.7 AU and +0.9 AU, respectively, p<0.001 for each). Adjusted differences for **hs-CRP** (+1.54 mg/L) and **IL-6** (+8.83 pg/mL) were also significant (p<0.001).

Correlation of Glycemic and Inflammatory Changes: Improvements in glycemic control in T2DM patients were moderately correlated with reductions in inflammatory markers. A decrease in HbA1c correlated with lower levels of mTORC1 and IL-6, indicating that better glycemic control is associated with significant anti-inflammatory effects. Notably, changes in IL-6 and CRP were strongly correlated, showcasing a systemic anti-inflammatory response. Furthermore, correlations among various free light chains (FLCs) were observed, particularly between Δκ and Δλ FLC, linked to their ratio.

Table 5. Pearson correlation matrix of changes (Δ) in biomarkers after metformin treatment (n = 22 patients).

Δ Variable	ΔHbA_{1c}	Δκ FLC	Δλ FLC	Δκ:λ Ratio	ΔmTORC1	ΔmTORC2	Δhs-CRP	ΔIL-6
ΔHbA_{1c}	1.000	0.360*	0.245	0.298	0.412**	0.334*	0.476**	0.523**
Δκ FLC	0.360*	1.000	0.587**	0.721**	0.445**	0.367*	0.502**	0.489**
Δλ FLC	0.245	0.587**	1.000	0.156	0.312*	0.289	0.378*	0.401*
Δκ:λ Ratio	0.298	0.721**	0.156	1.000	0.398*	0.325*	0.441**	0.456**
ΔmTORC1	0.412**	0.445**	0.312*	0.398*	1.000	0.678**	0.612**	0.589**
ΔmTORC2	0.334*	0.367*	0.289	0.325*	0.678**	1.000	0.534**	0.498**
Δhs-CRP	0.476**	0.502**	0.378*	0.441**	0.612**	0.534**	1.000	0.723**

Δ Variable	ΔHbA_{1c}	Δκ FLC	Δλ FLC	Δκ:λ Ratio	ΔmTORC1	ΔmTORC2	Δhs-CRP	ΔIL-6
ΔIL-6	0.523**	0.489**	0.401*	0.456**	0.589**	0.498**	0.723**	1.000

Significance: $p < 0.05$ *marked with* , $p < 0.01$ marked with *. *n = 22 patients with complete data. Note: Δ values are typically negative (because levels decreased); positive correlations indicate that larger decreases in one were associated with larger decreases in the other.*

Finally, a multiple linear regression was performed to identify independent predictors of the reduction in κ FLC (Δκ FLC as the outcome). We included ΔHbA_{1c}, ΔmTORC1, Δhs-CRP, ΔIL-6, baseline age, and baseline BMI as candidate predictors (these were chosen based on the correlation results and plausible influence). In this model (n=22), we found:

DISCUSSION

In this study, the impact of metformin on novel inflammatory biomarkers in sufferers with T2DM was evaluated. To our knowledge, this is one of the first studies to examine modifications in FLCs and mTOR signaling additives in T2DM patients receiving metformin. Our findings exhibit that a three-month course of metformin is not the most effective step forward in glycemic control but also produced widespread anti-inflammatory outcomes, as evidenced by reductions in FLCs, IL-6, CRP, and mTORC1/2 levels. These effects highlight an immunometabolic advantage of metformin in newly identified T2DM and underscore the intertwined nature of metabolic and inflammatory pathways in diabetes.

In alignment with previous research, the findings disclosed that newly diagnosed, drug-naïve T2DM patients had an upregulation of inflammatory profile compared to healthy individuals. The approximately 8–11 fold elevation in CRP and IL-6 levels in patients aligns with the concept that T2DM is a state of chronic inflammation (5, 18, 19). Elevated levels of IL-6 and CRP in diabetic patients have been reported to predict insulin resistance, dysfunction of β-cells, and the development of macro and microvascular complications (20-22). The current work extends these findings by showing that *polyclonal FLCs* are also regulated in T2DM. These outcomes support emerging evidence that the adaptive immune system is stimulated in metabolic disease (23-25). The elevated FLCs indicate increased B-cell activity or immunoglobulin production in T2DM, which may be driven by inflammatory cytokines (e.g., IL-6 is a potent B-cell stimulator). Notably, although both κ and λ FLCs were higher in diabetics, the κ/λ ratio remained mostly within normal limits, implying a broad, polyclonal activation rather than a skewed or clonal process. This is important because a normal ratio suggests the FLC elevation is due to inflammation and not a monoclonal gammopathy (26, 27). Indeed, a persistent abnormal κ/λ ratio might raise concern for plasma cell disorders, but in our T2DM patients, the ratio's modest increase likely reflects generalized immune activation, consistent with other inflammatory conditions (15). Furthermore, the results showed significantly higher mTORC1 and mTORC2 levels in T2DM patients at baseline, which provides clinical evidence for mTOR pathway upregulation in human diabetes. Hyperinsulinemia and nutrient excess in T2DM are known to activate mTORC1 in tissues as previously reported (28-30). The current data suggest that Peripheral Blood Mononuclear Cells (PBMCs) from diabetic patients have heightened mTOR complex formation or activity. This aligns with prior studies linking mTORC1 to insulin resistance and diabetic complications such as nephropathy (31). Elevated mTORC1 in immune cells can drive production of pro-inflammatory cytokines and antibody class switching (32, 33), potentially contributing to the inflammation we see in diabetes. Therefore, the baseline differences in our study reinforce the notion that T2DM involves significant immune and inflammatory perturbations involving both innate (CRP, IL-6, mTOR in myeloid cells) and adaptive (B-cell derived FLCs) immune pathways.

A key finding of this study is that metformin therapy was associated with broad anti-inflammatory changes. After 3 months, T2DM patients had markedly lower IL-6 and CRP levels. This is consistent with several clinical studies and meta-analyses showing that metformin can reduce circulating inflammatory markers in diabetes and other insulin-resistant states (34, 35). For example, Adeshara et al. (2020) observed that newly diagnosed T2DM patients on metformin for 3 months had reduced CRP and markers of oxidative stress, similar to our results (36). The reduction in IL-6 is particularly noteworthy because IL-6 is not only a marker but also a mediator of insulin resistance: high IL-6 levels promote hepatic glucose output and impair insulin signaling in muscle (37). By reducing IL-6, metformin may help break this vicious cycle of inflammation-induced insulin resistance (38, 39). Our data provides clinical confirmations of these anti-inflammatory actions.

Importantly, the findings of this study demonstrated

for the first time that metformin can remarkably reduce *FLCs* levels in T2DM patients. The ~29% drop in κ FLC and ~8% drop in λ FLC suggest that metformin dampened B-cell immunoglobulin production or promoted clearance of these immune products. This could be a downstream effect of lowering IL-6, since IL-6 drives B-cell differentiation into plasma cells and antibody production (including FLCs) (40). The normalization of the κ/λ ratio after treatment indicates that the polyclonal nature of the response was preserved, but the overall level of immunoglobulin free light chains receded toward normal. Prior research has identified elevated FLCs in chronic infections and autoimmune diseases, where effective treatment (antivirals, immunosuppressants) often leads to FLC declines (41). By analogy, our findings imply that controlling the underlying inflammatory/metabolic trigger in T2DM (with metformin) can mitigate this immunoglobulin overproduction. Although FLCs are not yet a routine diabetes biomarker, these results encourage further study into their role. If FLC reduction reflects improved immune health, they could serve as a novel indicator of the anti-inflammatory efficacy of therapies. Additionally, high baseline FLCs might identify patients with more pronounced inflammation who could benefit most from anti-inflammatory strategies. Furthermore, the current outcomes reported significant attenuation in mTORC1 and mTORC2 levels with metformin treatment. This agrees with the known molecular action of metformin: by stimulating AMPK, metformin indirectly inhibits the mTORC1 pathway (42, 43). Additionally, mTORC2 was also attenuated (~20%) by metformin in this study.

Despite significant improvements, IL-6 remained about sevenfold higher in patients than in controls, and mTORC1/2 levels were still significantly elevated. This incomplete resolution of inflammation has several possible explanations. First, 3 months may be too short to completely reverse long-standing inflammatory processes. Some pathways may require longer to reset; for example, reversal of immune cell phenotypes or clearance of accumulated inflammatory mediators might take more time. Indeed, our data suggests that immune activation markers (like IL-6, mTOR) lag glycemic improvements. Second, other sources of inflammation, such as adipose tissue in obesity, may not be fully addressed by metformin alone. Metformin typically causes only mild weight loss; patients with persistent obesity may continue to release inflammatory adipokines. In our study, patients did not have significant weight loss over 3 months (weight change data not shown), so adipose-driven inflammation likely persisted. Third, metformin's dose and monotherapy might limit the anti-inflammatory effect. Newer anti-diabetic medications such as GLP-1 receptor agonists and SGLT2 inhibitors have additional anti-inflammatory and weight-reducing properties (44, 45). Combining these with metformin or using more potent agents could produce greater normalization of inflammatory biomarkers. Our findings resonate with other studies that have noted partial reductions in inflammation with metabolic treatments, but not complete convergence to healthy levels (46). This suggests an "inflammatory memory" or irreversible component to the inflammation in T2DM, or simply that multi-faceted interventions are required.

Limitations

This study has several limitations that should be acknowledged. **First**, the sample size was modest (38 patients and 14 controls with complete data), and all patients were from a single center. While the study was sufficiently powered to detect within-patient changes, a larger multicenter sample would improve the generalizability of the findings. **Second**, the study design was a before–after comparison without a randomized control group for the treatment period. Although we included a healthy control group for baseline comparisons, all diabetic patients received metformin and there was no placebo group. Therefore, improvements in some markers could partly reflect the passage of time or behavioral changes (e.g. diet, exercise after diagnosis) rather than metformin per se. We attempted to minimize this by enrolling only newly diagnosed patients and providing standard lifestyle advice to all, but the **lack of randomization** means we cannot conclusively attribute all changes to metformin. Future randomized controlled trials (metformin vs placebo or vs other drugs) are needed to isolate the drug's specific effects (see Future Recommendations). **Third**, the follow-up duration was only 3 months. This relatively short period may not capture the full extent of metformin's impact on chronic inflammation or allow slower processes to manifest. Some biomarkers might continue to improve (or regress) with longer therapy.

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