

Levels of Myeloperoxidase, Malondialdehyde and Lipid Profile in Type 2 Diabetic Patients on Metformin Versus Glibenclamide Therapy

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ABSTRACT

Introduction: In diabetes mellitus, oxidative stress plays a major role in the development and progression of cardiovascular complications. Atherogenic index of plasma is a novel marker to predict the risk of atherosclerotic cardiovascular disease. Our study investigating the effects of metformin and glibenclamide on the levels of myeloperoxidase, malondialdehyde and lipid profile as well as identifying the correlations among these parameters in patients with type 2 diabetic mellitus. **Materials and Methods:** This study was carried out among 51 patients diagnosed with type 2 diabetic mellitus at Al-Wafaa Center in Mosul, between November 2019 and March 2020. We classified patients into three groups: newly diagnosed diabetic group, metformin receiving group and glibenclamide receiving group for up to 1 year. Then, plasma myeloperoxidase, malondialdehyde, atherogenic index and lipids levels were assessed. **Results:** Compared with glibenclamide, metformin receiving group revealed a significant reduction in myeloperoxidase, malondialdehyde, atherogenic index and lipid profile indices. The variation of plasma myeloperoxidase and malondialdehyde levels between metformin- and glibenclamide-receiving patients was statistically significant. However, the variation of atherogenic index and plasma lipid profile between treatment groups was non-significant. Moreover, serum myeloperoxidase level has been found to be positively correlated with malondialdehyde, low-density lipoprotein and total cholesterol in diabetic patients. As well, malondialdehyde bioavailability positively correlated with plasma triglyceride, very low-density lipoprotein levels and homeostatic model assessment of insulin resistance in glibenclamide treated patients. However, no statistically significant correlations have been found after using metformin. **Conclusion:** Metformin has cardio protective effects in diabetic patients, including reduced oxidative stress and improved dyslipidaemia, beyond its antihyperglycemic activity.

Keywords: Diabetes, Glibenclamide, Lipid profile, Metformin, Myeloperoxidase

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INTRODUCTION

Diabetes is a metabolic disease identified by chronic hyperglycaemia, resulting from impaired insulin secretion and/or insulin response.[1] These metabolic abnormalities can result in cellular changes with subsequent increase the risk of atherosclerotic cardiovascular disease.[2] Cardiovascular disease is a major cause of mortality and morbidity in type 2 diabetic mellitus (T2DM). Among many factors involved in the progression of atherosclerosis, elevated LDL-cholesterol levels are the major cause for atherosclerosis. In addition to other causes for atherosclerosis including smoking, impaired glucose tolerance, being-sedentary and overweight.[3]

Myeloperoxidase (MPO) is a peroxidase enzyme, most abundantly released by activated neutrophils. Many findings have been established that MPO may play a crucial role in the development of cardiovascular disease that may result from endothelial dysfunction and atherosclerotic plaque formation.[4, 5] In addition, elevated MPO level is associated with the risk of future cardiovascular diseases in apparently healthy individuals.[6] Malondialdehyde (MDA) is, an end-product of lipid peroxidation, found to be significantly higher in T2DM and is formed in atherosclerotic lesions.[7] In atherosclerosis, macrophages infiltrated in the arterial intima, forming thick plaques that can narrow the artery with a significant reduction in blood supply

which is the main cause of cardiovascular disease (CVD).[8]

Elevated levels of total cholesterol (TC) and LDL are well-established risk factors for CVD.[9] Patients with T2DM are more likely to have cardiovascular complications. Atherogenic index (AIP) is a critical marker for the risk of CVS, because of the direct correlation of AIP with atherosclerotic risk.[10] The glucose-lowering effect of metformin in the treatment of T2D is well-established. Many studies have suggested that metformin also improves the cardiovascular risk factors,[11-13] independently of its well-known glucose-lowering effects. These results suggest that metformin could reduce the risk of development of CVD by pleiotropic effects. [14] Despite being widely used in management of T2DM, there is growing concern regarding the vital roles of metformin in reduction of these inflammatory risk factors.

Glibenclamide (GLI) is the most commonly prescribed sulfonylurea agent in the management of T2DM. In addition to its glucose-lowering effects, a recent study in rats revealed that GLI plays a role in reducing the cardiovascular risk factors including MPO and MDA.[15] So far, the correlation between MPO, MDA and lipid profile in T2D on metformin or GLI therapy were not evaluated simultaneously. Hence, the present study aims to compare levels of MPO, MDA and lipids in patients with T2DM on metformin or GLI therapy, in addition to determine the associations among these parameters. The effects of

metformin and GLI on the concentration of MPO, MDA and AIP in patients with T2DM were evaluated to help to identify patients with cardiovascular risk factors and subsequent prevention of future development of CVS.

METHODS AND MATERIALS

Patients

Fifty-one patients with T2DM aged between 35 and 52 years, were enrolled in this comparative cross-sectional study from November 2019 to March 2020. This study was approved by the Research Ethics Committee of College of Pharmacy, University of Mosul. Informed consent was obtained from all participants before their inclusion into the study, and the whole study process was performed in accordance with the last update of Declaration of Helsinki. Patients were divided into three experimental groups, each with 17 patients: newly diagnosed (untreated) T2DM group, metformin receiving group (500 mg twice a day), and GLI receiving group (5 mg daily) for up to 1 year. Exclusion criteria involved patients on any other drugs, alcoholism and patients with diabetes complications or other clinical conditions were ineligible. Body mass index (BMI) was calculated for all patients by direct measurement of height and weight.

Laboratory analysis

Fasting blood samples were collected from patients after an overnight fasting. Immediately after incubation in water bath for 10 min, sera were separated in centrifuge at 3,500 rpm for 10 mins. Except for serum glucose, which was determined immediately, plasma samples for estimation MPO, MDA, triglyceride, HDL and cholesterol levels were stored at -20°C until used for analysis. LDL and VLDL were estimated by Friedewald's equation. AIP was

calculated as $\log (TG/HDL)$. [16] Fasting serum glucose and serum insulin were estimated by enzymatic colorimetric method and enzyme linked immunosorbent assay (ELISA), respectively. Then, serum insulin and glucose was used for insulin resistance calculation by using the following equation: [17]

$$HOMA-IR = \text{Insulin } (\mu\text{U/mL}) \times \text{Glucose (mmol/L)} / 22.5$$

Enzymatic myeloperoxidase activity was measured according to the following method [18] that depends on enzymatic oxidation of o-dianisidin (reducing substrate) in the presence of hydrogen peroxide (H₂O₂) to produce a coloured material which was measured at 450 nm. Serum MDA was measured by the modified method, [19] in which thiobarbituric acid (TBA) reacts with MDA forming a pink coloured compound, which is measured at 532nm. Lipid Indices were determined using colorimetric method depend on sulfo-phospho-vanillin reaction. [20]

Statistical Analysis

All data are expressed as mean ± standard deviation. Mann Whitney test and Kruskal-Wallis test followed by a Dunn's multiple comparisons test were used to analyse two or multiple datasets, respectively, using GraphPad Prism version 8.0 (San Diego, California, USA). The values $p < 0.05$ were regarded statistically significant.

RESULTS

Clinical characteristics of newly diagnosed and treated group

The mean duration of treatment, BMI and age in the study populations are given in Table I: Comparison of age, BMI and duration of treatment in study groups. There were non-significant variations between the untreated and treated diabetic groups.

Table I: Comparison of age, BMI and duration of treatment in study groups.

| Parameters | Untreated patients (n=17) | Metformin (n=17) | GLI (n=17) |
|-------------------------------|---------------------------|------------------|-------------|
| Age (years) | 43.46±6.614 | 46.22±5.214 | 43.72±5.121 |
| BMI (kg/m ²) | 26.22±1.098 | 25.33±0.912 | 24.99±0.243 |
| Duration of treatment (month) | - | 9.11±2.986 | 8.232±3.241 |

Validation of FSH, serum insulin and HOMA-IR

Serum glucose and HOMA-IR were significantly lower in metformin-receiving patients compared to untreated and GLI treated patients. In contrast, serum insulin decreased significantly in GLI-receiving patients as compared to other study groups (Table II).

Table II: Diabetic profile of study population.

| Parameters | Untreated patients | Metformin | GLI |
|--------------------------------|--------------------|-----------------------------|-----------------|
| Fasting serum glucose (mmol/l) | 12.01± 0.882 | 10.011±0.4232 a**** b** | 12.02±0.9231 |
| Insulin (µu/L) | 10.021±0.6228 | 9.024±0.4328 | 9.031±0.3856 a* |
| HOMA-IR | 5.101±0.4945 | 3.672±0.2245 a**** b**** | 4.358±0.2973 |

Values set as mean ± standard deviation. a represent variations between metformin and GLI-treated group in contrast to untreated patients; b represents variations between metformin and GLI groups. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$ represented statistically significant variations, as set by Kruskal-Wallis test followed by a Dunn's multiple comparison post-hoc test.

Validation of plasma MPO, MDA, AIP and lipid levels

Plasma MDA level in metformin treated group was significantly lower than that in newly diagnosed and GLI receiving group. Figure 1, 2 and table 3 showed that MPO, AIP, total cholesterol (TC) and LDL concentrations were significantly lower in metformin and GLI treated groups compared to untreated patients. Interestingly, plasma HDL

level was significantly higher in metformin and GLI receiving patients compared to newly diagnosed patients. However, no statistically significant variation was found between study groups for VLDL and TG (Table I).

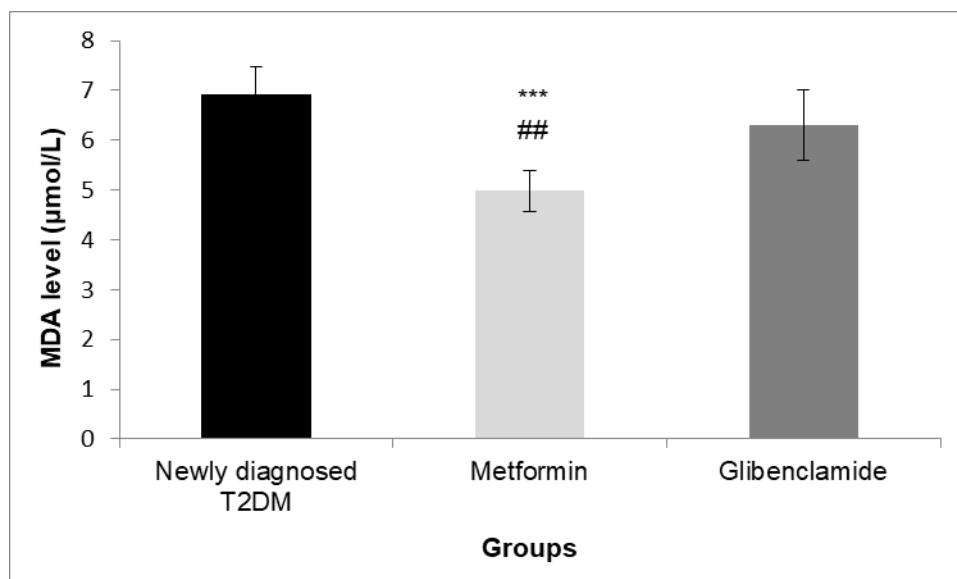


Figure 1: Comparative effect of metformin and GLI on serum malondialdehyde levels. Values expressed as mean \pm SD. Differences with a (***) $p < 0.001$ considered statistically significant when compared metformin and GLI with untreated type 2 diabetic patients; # represents statistically significant variations when compared metformin with GLI (## $p < 0.01$), as set by Kruskal-Wallis test followed by a Dunn's multiple comparison post-hoc test.

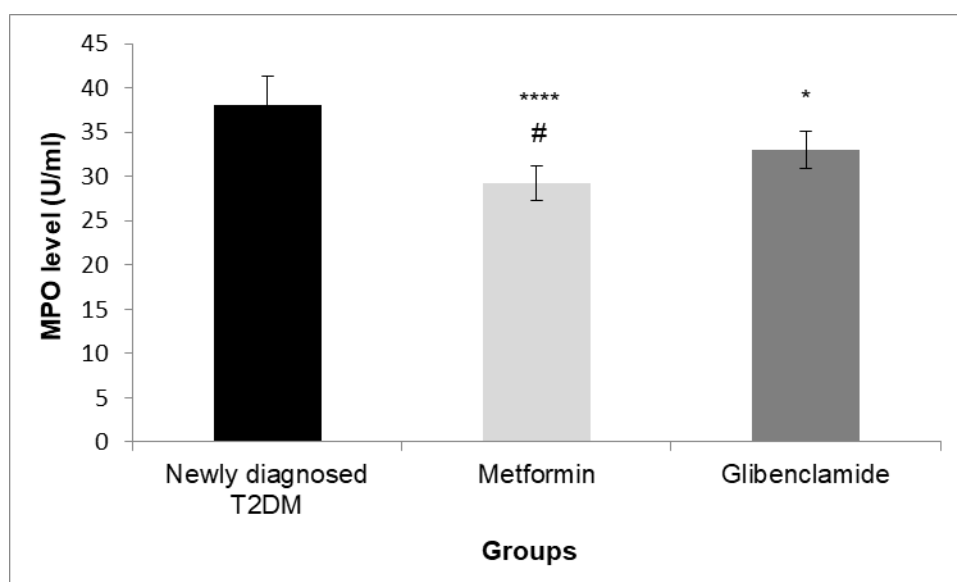


Figure 2: Comparative effect of metformin and GLI on serum myeloperoxidase levels. Values expressed as mean \pm SD. Differences with a (* $p < 0.1$; **** $p < 0.0001$) considered statistically significant when compared metformin and GLI with untreated type 2 diabetic patients; # represents statistically significant variations when compared metformin with GLI (#### $p < 0.0001$), as set by Kruskal-Wallis test followed by a Dunn's multiple comparison post-hoc test.

Table III: Lipid levels and AIP in study groups.

| Parameter | Newly diagnosed T2DM | Metformin | GLI |
|----------------------------|----------------------|------------------------------|----------------------------|
| Total cholesterol (mmol/L) | 6.68 ± 0.62 | 4.85 ± 0.6 ^{a****} | 5.57 ± 0.67 ^{a**} |
| LDL-c (mmol/L) | 4.46 ± 0.67 | 2.49 ± 0.63 ^{a****} | 3.32 ± 0.59 ^{a**} |
| HDL-c (mmol/L) | 0.81 ± 0.11 | 1.07 ± 0.14 ^{a****} | 0.99± 0.17 ^{a**} |
| Triglyceride (mmol/L) | 3.11 ± 0.52 | 2.83 ± 0.38 | 2.77 ± 0.4 |
| VLDL-c | 1.4 ± 0.24 | 1.29 ± 0.17 | 1.26 ± 0.18 |
| Atherogenic index | 0.58 ± 0.11 | 0.42 ± 0.08 ^{a***} | 0.45 ± 0.12 ^{a*} |

Data set as mean ± SD. ^a represents variations between metformin and GLI in comparison to untreated type 2 diabetic patients; ^b represents variations between metformin and GLI. Variations with a * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ represented statistically significant, as set by Kruskal-Wallis test followed by a Dunn's multiple comparison post-hoc test.

Table IV showed that plasma MDA levels were positively correlated with MPO, LDL and TC in patients with T2DM. Moreover, MDA bioavailability positively correlated with plasma TG, VLDL levels and HOMA-IR in GLI treated patients. However, no statistically significant correlations have been found after using metformin.

Table IV: Correlation between MDA and other parameters in study groups

| Parameters | MDA correlation coefficient (r) | |
|------------|---------------------------------|---------------|
| | Newly diagnosed | Glibenclamide |
| FSG | | |
| Insulin | | |
| HOMA-IR | | 0.5993* |
| TC | 0.5145* | |
| TG | | 0.5626* |
| LDL-c | 0.5049* | |
| HDL-c | | |
| VLDL-c | | 0.5626* |
| MDA | | |
| MPO | 0.5806* | |

* $p < 0.05$ represents statistically significant correlations.

DISCUSSION

The levels of MPO, MDA and lipid levels in type 2 diabetic patients on metformin versus GLI have not assessed yet. Findings of our study revealed that metformin reduced the cardiovascular risk factors more significantly than GLI, independently on insulin level variations. Although insulin level has been significantly higher in GLI treated patients, MPO and MDA concentrations have been significantly lower in metformin group with no significant variations on lipid profile.

The relationship between our overall parameters and cardiovascular risk factors is complex. According to this study, serum MPO level has been found to be closely associated with MDA, LDL and TC in type 2 diabetic patients. Moreover, MDA bioavailability positively correlated with plasma TG, VLDL levels and HOMA-IR in GLI treated patients. However, no statistically significant correlations have been found after using metformin.

One study has evaluated the correlation between MPO with MDA in type 2 diabetic patients. Shetty et al, reported

a positive association between MPO concentration and plasma MDA level which was in accordance with our findings.[21] Moreover, a study by Shiu et al, showed a positive correlation between MPO and carbamylated LDL in type 2 diabetic patients.[22] Despite the aforementioned findings, the correlation between the MPO and MDA in metformin treated patients is yet to be conclusively determined. Therefore, we evaluated a possible correlation between MPO, MDA and lipid profile in all study groups to clarify the contribution of these parameters as cardiovascular risk factors. However, no studies have been conducted to date to confirm the correlation between MDA and lipid levels in GLI-treated patients.

Many studies confirmed that plasma MPO and MDA levels were significantly increased in type 2 diabetic patients. [7, 10, 23, 24] Although MPO has a vital role in host defence against pathogen, but MPO produce oxidants involved in pathogenesis of atherosclerotic CVD through induction of endothelial dysfunction, conversion of physiologically

functional HDL and LDL to dysfunctional HDL and atherogenic modified LDL, respectively, induction of endothelial cell damage and expression of tissue factor involved in atherosclerotic plaque formation. [25] The intermolecular cross-links induced by MDA are involved in the pathogenesis of late complications of DM, resulting in increased stiffness of cardiovascular tissue. [10]

When activated by increased glucose levels, diabetic neutrophils showed higher MPO expression. Moreover, there is a well-known association between risk for CVS and increased serum MPO levels. Nevertheless, the role of MPO in development of diabetic complications is not clear. Thus, the current study revealed that serum MPO levels were significantly reduced in plasma of patients treated with metformin and GLI compared to the newly diagnosed diabetic patients, which is in line with other findings. [15, 26] Nevertheless, Kolahian *et al.* revealed no changes in MPO concentration in GLI-treated diabetic animals. In addition, Jellic-Knezovic *et al.* showed that metformin has no effect on MPO concentration in T2D patients. [27] Dyslipidaemia associated with T2DM are attributed to defect of insulin actions and hyperglycemia. [28] Many studies shows discrepant results concerning metformin and GLI effects on lipid profile. [29, 30] Many results, in line with ours, showed that serum LDL and TC were significantly reduced with concomitant significant increased levels of serum HDL in metformin and GLI treated patients compared to untreated type 2 diabetic patients. [30-32] whereas others reported only increase in HDL with GLI or decrease in TC with metformin. [33, 34] Still many previous researches reported no significant changes in lipid profile. [35-37] It is not clear as to why there is a variation between the metformin and GLI effects on lipid profile and further studies are required to clarify this issue. However, a meta-analysis covering forty-one independent clinical trials suggested that metformin reduce LDL and TC significantly, with no effect on TG and HDL levels. [29] Our findings, except HDL cholesterol, are in accordance with these results. Diabetes is correlated with endothelial dysfunction, which in turn may associated with systemic insulin resistance and precede the development of diabetes. [38, 39] Interestingly, HDL has been reported to induce eNOS/NO mediated vasorelaxation. [40] In addition, a recent study by Vaisar *et al.* established the direct stimulatory effect of HDL on eNOS expression and activation in primary aortic endothelial cells. [41] Accordingly, this is probably the possible explanation for the cardio protective effect of metformin in type 2 diabetic patients.

CONCLUSION

By assessing levels of serum MPO, MDA, AIP and lipids helps to reduce risk for CVS and diabetic complications. Levels of serum MPO, MDA and lipids were significantly reduced in type 2 diabetic patients treated with metformin and GLI. Moreover, reduced level of MPO significantly associates with lower concentrations of MDA, LDL, AIP and TC in type 2 diabetic patients. This confirms the fact that metformin and GLI offer antioxidant effect along with glycaemic control, thus providing protection for pancreas from oxidative damage throughout development of diabetic complications.

CONFLICT OF INTEREST

The author declare that they have no conflicts of Interest

AUTHORS' CONTRIBUTION

All authors have made contribution to this work.

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