Research Article

Comparative effects of metformin and glibenclamide on the redox balance in type 2 diabetic patients

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Abstract

It is known that there is a strong association between oxidative stress and insulin resistance in type 2 diabetes mellitus (T2DM). Although the role of glibenclamide in diabetes treatment has been evaluated, there is only limited evidence about its antioxidant effects in diabetic patients. Moreover, previous studies showed discrepant results regarding the effects of metformin on antioxidant/ oxidant parameters in type 2 diabetic patients. The present study aimed to evaluate the effects of metformin versus glibenclamide on oxidative stress biomarkers, represented by serum malondialdehyde (MDA), nonenzymatic, and enzymatic antioxidants in type 2 diabetic patients. Forty-six patients with T2DM participated in this study and categorized into 3 groups, Group A included 17 newly diagnosed diabetic patients, group B included 15 diabetic patients received metformin monotherapy (1000 mg/day) for up to 1 year and group C included 14 diabetic patients received glibenclamide monotherapy (5 mg/day) for up to 1 year. Serum MDA, catalase (CAT), vitamin C, E, and reduced glutathione (GSH) were measured. We found significantly lower concentrations of MDA and significantly higher antioxidant levels (CAT, GSH, vitamin C, and E) in the metformin-treated group compared to the glibenclamide counterpart. Our data confirmed that metformin has a more beneficial effect on oxidant/antioxidant status compared to glibenclamide, therefore, provides protection against reactive oxygen species (ROS) induced oxidative damage during diabetes.

Keywords

Introduction

Type 2 diabetes mellitus (T2DM), a chronic metabolic disorder, makes up over 90% of all cases of diabetes and it is characterized by hyperglycemia due to disturbances in insulin synthesis and efficiency (Taylor 1999). It is also commonly associated with dyslipidemia, hypertension, atherosclerosis (Choi and Ho 2018), and exacerbated oxidative stress (Rovira-Llopis et al. 2013).

Oxidative stress reflects a disturbance in the balance between reactive oxygen species (ROS) production and the antioxidant defense mechanisms (Hernandez-Mijares et al. 2013). In biological systems, antioxidants provide protection against ROS by various scavenging mechanisms and include both enzymatic (superoxide dismutase, catalase (CAT) and glutathione peroxidase) and nonenzymatic mechanisms (Vitamin C, E and reduced glutathione (GSH)) (Mansourian et al. 2018). It has been found that

chronic hyperglycemia results in overproduction of ROS through auto-oxidation of glucose and production of advanced glycation end products which in turn is associated with the development, and progression of T2DM and its complications (Paneni et al. 2013). Moreover, enhanced generation of ROS in T2DM has been shown to result in hyperinsulinemia, insulin resistance (Aouacheri et al. 2015), and biomolecules oxidation (Cnop et al. 2005).

The cornerstones of diabetes management include lifestyle modifications in combination with pharmacological therapy. Sulfonylureas, biguanides, meglitinides and thiazolidinediones are currently available oral antidiabetic agents that can improve glycemic control either in combination or as individual agent (Choi and Ho 2018). The main biguanide member, metformin, represents one of the most widely prescribed antihyperglycaemic agents (Emami-Riedmaier et al. 2015). Its action is by improving insulin sensitivity and inhibition of gluconeogenesis which in turn leads to an increase of glucose uptake in skeletal muscle and adipocytes and reduces hepatic glucose output, respectively (Kirpichnikov et al. 2002). In addition, metformin has been reported to have a weight reduction and lipid-lowering effects (Scarpello and Howlett 2008). Whereas glibenclamide, a sulfonylurea oral hypoglycemic drug, stimulates insulin secretion from the existing beta cells of the pancreas through inhibition of ATP-sensitive K^+ channels which in turn reduces hepatic glucose production (Gao et al. 2017).

Oxidative stress has a crucial contribution in the development of diabetes and its complication (Saravanan and Pari 2006). However, most available antioxidants and vitamins did not demonstrate any clinical benefits or consistent results (Cuzzocrea et al. 2001; Pazdro and Burgess 2010). Therefore, the roles of oral hypoglycemic agents in lowering oxidative stress need to be elucidated.

To our knowledge, there is only a little information about the antioxidant effects of glibenclamide in type 2 diabetic patients. Moreover, previous clinical studies of metformin on the oxidant /antioxidant balance have yielded conflicting results (Faure et al. 1999; Signorini et al. 2002; Abdulkadir and Thanoon 2012). So, the present study aimed to investigate the effects of metformin versus glibenclamide on oxidative stress biomarker, represented by serum malondialdehyde (MDA), nonenzymatic and enzymatic antioxidants levels in patients with T2DM.

Materials and methods

Instrumentation

UV-VIS Spectrophotometer PD-303 UV (APEL brand, Japan) and ELx 800 Universal Microplate Reader (BioTek, USA) were used.

Chemicals and reagents

Thiobarbituric acid (TBA) was obtained from (Cayman, USA). Metformin (Siofor 500) was purchased from Berlin Chemie (Germany). Glibenclamide (Glibesyn) was obtained from, Medochemie (Cyprus). FSG was determined by kit purchased from BIOLABO (France). Insulin AccuBind ELISA Kit was obtained from Monobind (USA). All other chemicals were purchased from Sigma Aldrich (USA).

Study designs and subjects

A retrospective cross-sectional study was conducted on patients with T2DM in Al-Waffaa Centre of Diabetes Management and Research, Mosul, Iraq. It was done between October, 2019 and January, 2020. This study included 46 patients with T2DM of both sexes aged between 32 and 56 years. It 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 the Declaration of Helsinki. The participants were categorized into three groups, Group A included 17 newly diagnosed diabetic patients, group B included 15 patients received metformin monotherapy (1000 mg/day) for up to 1 year and group C included 14 patients received glibenclamide monotherapy (5 mg/day) for up to 1 year. Diagnosis of T2DM was made according to the World Health Organization and American Diabetic Association criteria. Pregnant and lactating women, patients receiving additional drugs, vitamins, or supplements, patients with medical conditions other than type 2 diabetes, alcoholics, smokers, and patients who underwent medication changes during the period of treatment were excluded from the study. Anthropometric variables such as height and weight were obtained to calculate the body mass index (BMI).

Sample collection and biochemical assay

Venous blood samples were collected from each diabetic patient after overnight fasting in plain tubes. Following an incubation period of 10 min in a water bath at 37 °C, sera were separated by centrifugation at 4,000× g for 10 mins and then aliquoted and stored at -20 °C to be analyzed later, except for serum glucose level which was estimated immediately.

Estimation of serum glucose, serum insulin, and insulin resistance

Fasting serum glucose (FSG) was estimated by the enzymatic colorimetric method and the absorbance was measured at 505 nm. Serum insulin was measured by enzyme-linked immunosorbent assay (ELISA) and the absorbance was determined at 450 nm. Insulin resistance was assessed by the homeostatic model assessment (HOMA-IR) according to the following equation:

$$\text{HOMA-IR} = \text{Insulin (micro units (}\mu U)\text{ / mL)} \times \text{Glucose (mmol/L) / 22.5 (Matthews et al. 1985)}$$

Estimation of serum malondialdehyde

Serum MDA was evaluated by the modified method,(Guidet and Shah 1989) in which MDA reacts with thiobarbituric acid to form a colored product useful in the determination of lipid peroxidation at 532nm.

Estimation of serum catalase activity

CAT activity in serum was determined by measuring the decrease in hydrogen peroxide absorbance at 240 nm wavelength using the spectrophotometric method (Aebi 1984).

Estimation of serum reduced glutathione concentration

GSH in serum was estimated according to the modified standard Ellman method (Sedlak and Lindsay 1968), where a reaction between GSH and 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB, Ellman's reagent) leads to the formation of an intensely yellow compound which was measured at 412 nm.

Estimation of serum vitamin c and vitamin e concentrations

Vitamin C in serum was determined by measuring a decrease in the absorption of 2,6-dichlorophenolindophenol at 520 nm (Omaye et al. 1979). Serum Vitamin E was determined by measuring the absorbance of ferrous ions, produced by the reduction of ferric iron, at 460 and 520 nm (Bukovits and Lezerovich 1987).

Statistical analysis

Mann Whitney test and Kruskal-Wallis test followed by a Dunn's multiple comparisons test were performed to compare two or multiple datasets, respectively. Spearman rank correlation analysis was used to determine associations between MDA with other laboratory parameters. All values were expressed as mean±SD and statistical significance was established when p < 0.05. All analyses were conducted using Graphpad prism software version 8.0 (San Diego, California, USA).

Results

Demographic characteristics of newly diagnosed and treated diabetic groups

Table 1 shows the age, BMI, and duration of treatment in the treated and newly diagnosed diabetic groups. There were no significant differences between the groups.

Table 1. Demographic characteristics of the studied groups.

Parameter(unit)	Newly diagnosed T2DM	Metformin	Glibenclamide
Age (years)	40.47±6.634	43.07±7.245	41.79±7.029
BMI (kg/m^2)	25.07±1.167	24.59±0.9819	24.54±1.201
Duration of treatment (months)	–	7.100±3.146	7.286±2.847

Effect on HOMA-IR, serum insulin, and glucose:

Metformin treated group showed significantly lower FSG level and HOMA-IR index compared to the untreated and glibenclamide treated patients. However, a significant elevation in insulin level was observed in the glibenclamide treated patients compared to other groups (Table 2).

Table 2. Diabetic profile of the studied groups.

| Parameter(unit)	Newly diagnosed T2DM	Metformin	Glibenclamide
FSG (mmol/l)	12.21± 1.064	8.787±0.5167 [a****, b**]	10.88±0.7934
Insulin(μμ/L)	8.741±0.7289	9.167±0.4835	9.393±0.5240 [a*]
HOMA-IR	4.732±0.4748	3.574±0.1834 [a****, b****]	4.530±0.2562

The results are expressed as mean±SD. [a] comparing metformin and glibenclamide treated groups in contrast to newly diagnosed one; [b] comparing between metformin and glibenclamide treated groups. *indicates statistically significant differences, as determined by Kruskal-Wallis test followed by a Dunn's multiple comparisons post-hoc test (*p < 0.05; **p < 0.01; ****p < 0.0001).

Effect on oxidant–antioxidant parameters

It was observed that the MDA level was significantly decreased in the metformin- treated patients compared to glibenclamide and untreated groups (Figure 1). Figure 2 showed that serum levels of GSH, vitamin C and vitamin E were significantly higher in metformin treated group compared to the other groups.

Moreover, the result analysis revealed that both metformin and glibenclamide treated groups have significantly higher catalase levels compared to the untreated group. However, metformin resulted in a significantly higher catalase level than glibenclamide (Figure 3).

The association of MDA with other laboratory parameters in metformin-treated group has been examined. MDA showed a significant negative correlation with vitamin C and vitamin E (Table 3).
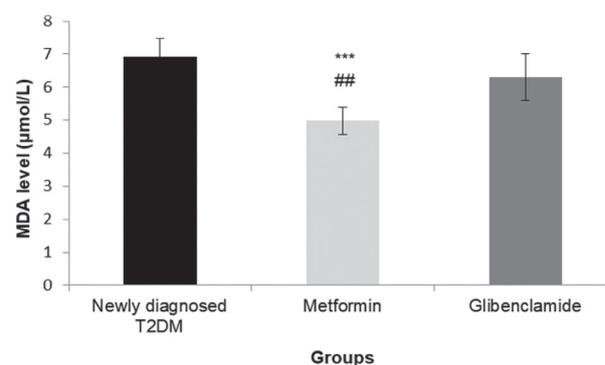
Figure 1. Effects of metformin and glibenclamide on serum MDA level. The results are expressed as mean±SD.* indicates statistically significant differences in contrast to newly diagnosed group (***p < 0.001); # indicates statistically significant differences between treated groups (##p < 0.01), as determined by Kruskal-Wallis test followed by a Dunn's multiple comparisons post-hoc test.
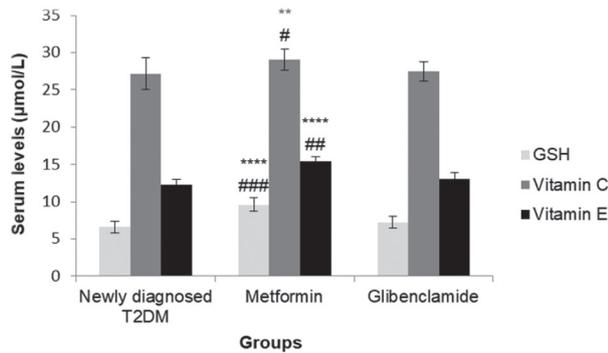
Figure 2. Effects of metformin and glibenclamide on serum levels GSH, vitamin C and vitamin E. The results are expressed as mean±SD. * indicates statistically significant differences in contrast to newly diagnosed group (**p < 0.01; ****p < 0.0001); # indicates statistically significant differences between treated groups (#p < 0.05; ##p < 0.01; ###p < 0.001), as determined by Kruskal-Wallis test followed by a Dunn's multiple comparisons post-hoc test.
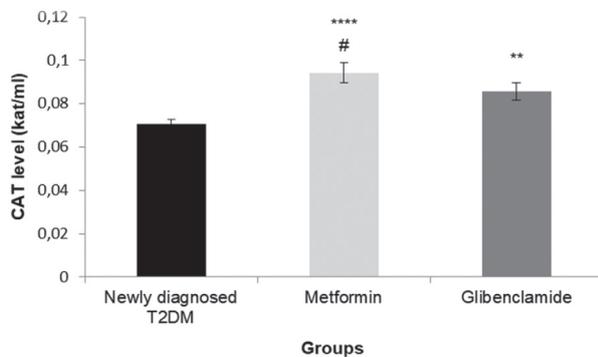
Figure 3. Effects of metformin and glibenclamide on serum catalase level. The results are expressed as mean±SD. * indicates statistically significant differences in contrast to newly diagnosed group (**p < 0.01; ****p < 0.0001); # indicates statistically significant differences between treated groups (#p < 0.01), as determined by Kruskal-Wallis test followed by a Dunn's multiple comparisons post-hoc test.

Table 3. Spearman correlation of MDA with variable parameters in metformin treated group.

Parameters	MDA correlation coefficient (r)
FSG	0.1792
Insulin	0.3487
HOMA-IR	0.4991
CAT	0.09863
GSH	-0.1382
Vitamin C	-0.5267*
Vitamin E	-0.6387*

*indicates statistically significant correlation, as determined by Spearman test (*p < 0.05).

Discussion

While metformin and glibenclamide have frequently been used in T2DM, studies focused on their effects on oxidative stress have shown discrepant results. The present study was done to assess the effects of metformin and gli-

benclamide on lipid peroxidation and antioxidant level in type 2 diabetic patients. In this study, although metformin exhibited a nonsignificant increase in insulin production, FSG, and HOMA-IR level were found significantly lower compared to the glibenclamide and untreated group (Table 2). Metformin possibly exerts an antihyperglycemic effect through improving insulin sensitivity in diabetic patients. These outcomes are in accordance with the research of Maithili Karpaga Selvi et al. (2015) and Yin et al. (2008).

Depletion of antioxidants and increased lipid peroxidation are features of diabetes mellitus (Chandirasegaran et al. 2018). Lipid peroxidation is a process that occurs due to a reaction between ROS and lipids resulting in oxidative damage of cellular membrane lipids and has been implicated in a wide range of diseases including diabetes (Dalle-Donne et al. 2006). A secondary product of lipid peroxidation, MDA, is used as an indicator of oxidative stress (Ayala et al. 2014). In our study, metformin brought about a significant decrease in the level of MDA compared to glibenclamide and untreated diabetics (Figure 1) supporting the idea that metformin provides protection against lipid peroxidation and oxidative damage in diabetes (Chukwunonso Obi et al. 2016a) which could be as a result of improved antioxidants' status. This finding was in concomitant with the studies of Abdulkadir and Thanoon (2012) and Chukwunonso Obi et al. (2016b). The inhibition of mitochondrial complex I by metformin treatment may contribute to the lower MDA level through attenuation of ROS production (Marchetti et al. 2004). However, Skrha et al. (2007) showed a significant increase in MDA level after 3 months of metformin therapy in T2DM patients. They concluded the association of metformin treatment in such patients with activation of oxidative stress. In another study, metformin revealed a nonsignificant reduction in MDA levels (AA et al. 2017).

Various enzymatic and nonenzymatic antioxidants are produced in the body to counteract oxidative stress through detoxification of ROS (Correia et al. 2008). Our present finding demonstrated that metformin-treated patients have significantly higher levels of catalase, GSH, vitamin C, and vitamin E compared to the newly diagnosed and glibenclamide treated groups (Figures 2, 3). The reductions observed in the enzymatic and nonenzymatic antioxidant levels in the newly diagnosed and glibenclamide groups suggest their excessive utilization in reducing ROS, as demonstrated by increased lipid peroxidation, induced by hyperglycemia (Subash-Babu et al. 2014). This observation is consistent with previous studies (Banik et al. 2018; Maithili Karpaga Selvi et al. 2015). Although glibenclamide group showed a higher catalase level compared to untreated patients, no favorable effect has been found on the MDA level. This reveals the reduced antioxidant potential of this antidiabetic medicine. In contrast, metformin-treated group showed an increase in the antioxidant levels which is in agreement with previous studies (Pavlovic et al. 2000; Skrha et al. 2007). This improvement could be related to the correction in hyperglycemia and lipid peroxidation which in turn results in a decrease in

antioxidants (enzymatic and nonenzymatic) utilization. These results support the idea that oxidant-antioxidant imbalance induced by type 2 diabetes can be counteracted by metformin. Moreover, this beneficial antioxidant effect might be responsible for improved insulin sensitivity in diabetic patients after metformin administration.

On Spearman's correlation analysis, FSG, insulin, and HOMA-IR showed a nonsignificant relationship with MDA and antioxidants in metformin-treated group (data not shown), whereas a significant negative correlation has been found between MDA and nonenzymatic antioxidants (vitamin C and vitamin E) in this group (Table 3), indicating the decrease in lipid peroxidation with increased antioxidant levels in metformin-treated patients compared to glibenclamide and untreated groups where increased lipid peroxidation resulted in depletion of endogenous antioxidants. So, improvement of the antioxidant defense system by metformin may be due to its antioxidant nature that prevents lipid peroxidation and scavenges free radicals.

It should be noted that our study has some limitations. Glycated hemoglobin (HbA1c) was not measured. Mo-

reover, the number of diabetic patients participate in this study was low. Other studies on larger sample sizes are recommended for more confirmation.

Conclusion

The present study revealed that metformin monotherapy was more effective in ameliorating oxidative stress and improving the antioxidant defense systems compared to glibenclamide in type 2 diabetic patients, thus provide protection against oxidative stress-induced injury during diabetes and its complications.

Acknowledgements

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