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Research Article

Assessment of the effects of metformin and glibenclamide on the concentration of selected trace elements in type 2 diabetic patients

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Abstract

Metformin and glibenclamide may have beneficial effects on the levels of trace elements in diabetic patients. The aim of the current study was to assess the effects of metformin and glibenclamide on the concentrations of copper (Cu), zinc (Zn) and magnesium (Mg) in patients with type 2 diabetes mellitus. The metformin-treated patients showed significantly lower serum Cu levels compared with the untreated and glibenclamide groups. In addition, treatment with metformin was associated with a significant increase in serum concentrations of Zn compared to the newly diagnosed patients, whereas it did not show a noticeable alteration in the serum level of Mg. In contrast, the glibenclamide treated group revealed significantly higher Zn and Mg levels compared with the newly diagnosed group, while the serum level of Cu was not significantly modified. In conclusion, treatment with metformin led to a reduction in serum Cu and an increase in serum Zn concentrations, whereas glibenclamide treatment displayed enhancement in serum Zn and Mg levels.

Keywords

Copper, Glibenclamide, Magnesium, Metformin, Zinc

Introduction

Trace elements are divalent cations that play essential roles in the human body. These cations, including copper (Cu), zinc (Zn) and magnesium (Mg), are necessary for different cellular functions and the alterations in their concentrations can contribute to the development of many diseases, such as T2DM (Dosa et al., 2011, Viktorinova et al., 2009). Cu and Zn have a crucial role in the mechanisms of oxidation-reduction reactions. Cu has both prooxidant and antioxidant effects through the generation of free radicals and catalyzation of the superoxide dismutase enzyme, which protects cells from superoxide radicals, respectively. In contrast, Zn has only an antioxidant effect by preventing reactive oxygen species from causing damage to proteins and enzymes (DiSilvestro, 2000). So, an oxidant/antioxidant imbalance can result from a disturbance in the level of these elements, which in turn induces diabetes and its complications (Soinio et al., 2007). In addition, low Zn levels can negatively affect the production and secretion of

insulin from the islet cells of the pancreas (Quraishi et al., 2005). Mg is another important trace element that has a vital role in glucose homeostasis. It has been found that its deficiency can contribute to decreased insulin sensitivity and secretion (Huerta et al., 2005).

Although numerous studies have been carried out to determine the serum levels of trace elements in type 2 diabetic patients receiving metformin, contradictory results have been obtained (Chakraborty et al., 2011, Peters et al., 2013). Moreover, little known data is available on the effect of glibenclamide on the concentrations of various divalent cations in type 2 diabetic patients. Hence, taking into consideration the aforementioned findings, the present study was performed to investigate the effects of metformin versus glibenclamide on the concentrations of some trace elements (Cu, Zn, and Mg) in T2DM patients.

Materials and methods

Subjects

This was a case-control retrospective study that included 64 subjects (controls and diabetics of both genders) aged between 28 and 56 years. Patients with T2DM were diagnosed and recruited at the Al-Waffaa Diabetes Management and Research Centre, Mosul, Iraq, between August-December, 2019. This study was authorised by the Committee of Research Ethics of the University of Mosul, Pharmacy College. Prior to their participation in the study, informed permission was received from all participants and the study process was carried out in line with the latest update of the Helsinki Declaration. Patient group, comprised of 48 individuals with T2DM, was categorized into three groups (16 patients each). Group B included newly diagnosed diabetic patients, group C included patients already treated with metformin (SioforR, Berlin Chemie) 500 mg two times per day for a 3–12 months period, and group D included patients already receiving glibenclamide (GlibesynR, Medochemie) 2.5 mg two times per day for the same period as group C. The control group (Group A) included healthy subjects with age matching to the patient group. Patients complaining of any systemic disease other than type 2 diabetes, pregnant and lactating mothers, alcoholics, smokers, patients taking drugs other than metformin or glibenclamide, or those receiving vitamins or minerals, and those who have undergone treatment modifications during the treatment period have not been included in the study. The body mass index (BMI) has been calculated according to the height and weight of all subjects.

Sample collection

Following an overnight fast, blood samples were drawn from all participants and collected in plain tubes. After 10 minutes of incubation in a water bath at 37 °C, samples were centrifuged at 4,000x g for 10 minutes to separate serum and then stored at -20 °C until measurement.

Evaluation of serum glucose and insulin

The concentration of fasting serum glucose (FSG) was assessed by an enzymatic colorimetric method at 505nm absorbance. The level of serum insulin was determined at 450nm absorbance using the enzyme linked immunosorbent assay (ELISA) technique.

Estimation of serum copper, zinc, and magnesium

An atomic absorption spectrophotometer (Shimadzu AA-670, Kyoto, Japan) was used to determine Cu, Zn, and Mg in serum. Prior to analysis, serum samples of Cu, Zn, and Mg were diluted with deionized water with a dilution factor of 1, 5 and 50, respectively. Different concentrations (0.1–2.5 ppm) of Cu, Zn and Mg solutions were used for system calibration. The absorbance of Cu, Zn, and Mg was measured at 224.8, 213.9, and 285.2 nm, respectively.

Statistical analysis

All values were shown as mean ± SD. The Kruskal-Wallis test followed by the Dunn's multiple comparison test were used for multiple comparisons. $P < 0.05$ was considered a statistically significant difference. Statistical analyses were performed using GraphPad Prism software version 8.0 (San Diego, California, USA).

Results

Demographic characteristics of the study groups:

Age, duration of diabetes and BMI of diabetic patients and controls are shown in Table 1. Non-significant differences were found between the groups.

Estimation of serum insulin and glucose concentrations

Table 1. Demographic characteristics of diabetic patients and control subjects.

Parameters	Control	Newly diagnosed	Metformin	Glibenclamide
Age (years)	41.9 ± 8.43	41 ± 7.6	44.1 ± 6.3	42.88 ± 6.6
BMI (kg/m^2)	26.3 ± 2.9	26.1 ± 0.5	25.65 ± 0.8	25.64 ± 1.1
Duration of treatment (months)	–	–	6.9 ± 2.8	6.8 ± 3

Table (2) shows the FSG and insulin of all the participating groups. It has been found that serum glucose concentration was significantly higher in the newly diagnosed and glibenclamide treated groups compared to the control group. In contrast, the metformin treated group exhibited a comparable FSG level to that of the control group and

significantly lower as compared to the newly diagnosed diabetics. However, the insulin level was found to be significantly lower in metformin and newly diagnosed patients compared to the control group, while the glibenclamide treated and control groups revealed comparable levels.

Estimation of serum concentrations of copper, zinc, and magnesium

Table 2. Estimation of serum glucose and insulin of participating individuals.

Parameters	Control	Newly diagnosed	Metformin	Glibenclamide
FSG (mmol/l)	5.3 ± 0.4	11.9 ± 1.4 ^{a****}	9.1 ± 0.2 ^{b****}	11 ± 0.8 ^{a****}
Insulin(µu/L)	10.5 ± 1.3	8.2 ± 0.9 ^{a****}	8.9 ± 0.7 ^{a*}	9.5 ± 0.4

Data is presented as mean ± SD. [a] Comparison versus control; [b] Comparison versus newly diagnosed group. Statistically significant differences were evaluated by the Kruskal-Wallis and a Dunn's multiple comparisons post-hoc test (*$p < 0.05$; ****$p < 0.0001$).

Our data showed that serum concentrations of Cu were significantly higher in the glibenclamide and untreated diabetic patients compared to the control group. In contrast, treatment with metformin led to a decrease in serum Cu level to become comparable with that of the control group and significantly lower compared with the glibenclamide treated and newly diagnosed groups (Figure 1A). As shown in Figure 1B, both glibenclamide treated and the control groups have comparable serum Zn concentrations, whereas metformin treated and newly diagnosed patients have significantly lower Zn levels as compared with the control. However, treatment with metformin and glibenclamide significantly increased serum Zn levels compared to the newly diagnosed group. Concerning serum Mg, the glibenclamide and control groups showed a comparable level, whereas the metformin treated and newly diagnosed groups showed significantly lower levels compared with the control. Although glibenclamide treated patients revealed significantly higher Mg levels compared with the newly diagnosed diabetics, there were no significant differences in comparison with the metformin treated group (Figure 1C).

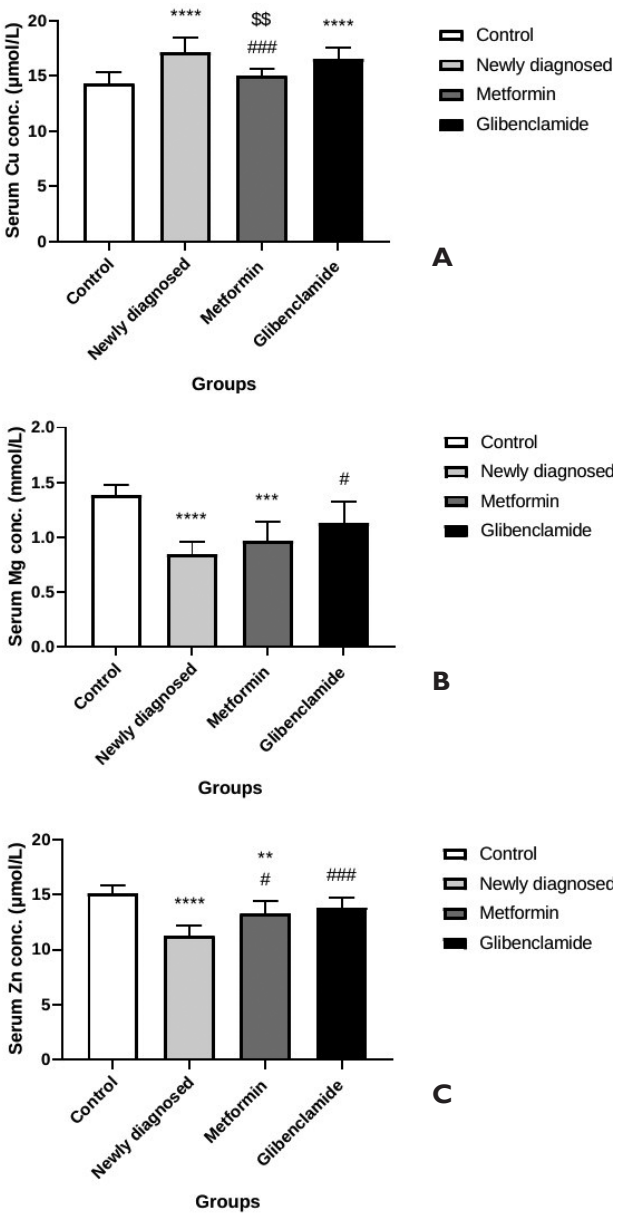

Figure 1. Effects of metformin versus glibenclamide on serum concentrations of A) Cu, B) Zn, and C) Mg in type 2 diabetic patients.* indicates statistically significant differences compared to the control group (**p < 0.01; ***p < 0.001; ****p < 0.0001); # indicates statistically significant differences compared to the newly diagnosed group (#p < 0.05; ###p < 0.001); $ indicates statistically significant differences between the metformin and glibenclamide treated groups, as determined by the Kruskal-Wallis test followed by a Dunn's multiple comparisons post-hoc test.

Discussion

While the role of glibenclamide and metformin in the treatment of diabetes has been well assessed, little evidence about their activity on trace elements in type 2 diabetic patients exists. This study aimed to investigate the effects of glibenclamide and metformin on Cu, Zn and Mg levels in type 2 diabetic patients.

The findings of the current study revealed that newly diagnosed diabetic patients have much higher Cu levels and lower Zn and Mg levels than healthy controls. In addition, newly diagnosed diabetic patients showed higher FSG and lower insulin levels compared to the control. These results were consistent with previously reported studies, indicating the impact of these trace elements on the pathogenesis of T2DM as well as the effect of glycemic status on their levels (Khan et al., 2015, Viktorinova et al., 2009). However, Basaki et al. (2012) showed that diabetic patients have a lower Cu level compared to the control, whereas Terrés-Martos et al. (1998) exhibited no significant differences in the Cu level between diabetic patients and the control. Higher levels of Cu in type 2 diabetic patients are possibly associated with hyperglycemia due to disruption of the Cu-binding

proteins (Talaei et al., 2011), whereas lower levels of Zn and Mg may be attributed to hyperglycemia induced disturbances in the factors that maintain their balance, such as intestinal absorption and urinary excretion (Mishra and Mishra, 2017, Yerlikaya et al., 2013).

The present study showed that patients treated with metformin have a significantly lower serum Cu level compared with glibenclamide and newly diagnosed diabetic patients, while treatment with glibenclamide had no significant effect. Our results are inconsistent with those of Dosa et al. (2011), who reported a non-significant effect of metformin on the plasma concentration of Cu in type 2 diabetic patients. In contrast, our data is in line with Zargar et al. (1998), who revealed that glibenclamide does not affect plasma copper levels. In the current study, the difference between the effects of the treated groups on serum Cu may be related to glycaemic control. Metformin treated patients showed a significantly lower FSG level compared to the newly diagnosed group. This could be, in part, explained by the increasing affinity of Cu for ceruloplasmin due to decreased glycation by metformin (Roxborough et al., 1995, Sarkar et al., 2010). However, the results of our study showed no difference between the metformin and glibenclamide treated groups in terms of their effects on FSG. Another reason for a decrease in serum Cu may be due to the ability of metformin to abstract Cu ions from the biological system, resulting in complex formation (Quan et al., 2015).

With respect to Zn, both the metformin and glibenclamide treated groups showed significantly higher levels compared to the newly diagnosed group. Previous studies about the effect of metformin on the serum Zn level in type 2 diabetic patients have shown conflicting and even contrary results. Dosa et al. (2011) showed that metformin administration does not lead to significant changes in the plasma concentration of Zn in patients with non-insulin-dependent diabetes mellitus. In contrast, Naik et al. (2019) revealed that metformin alone or in combination with glimiperide causes a significant decrease in serum zinc levels in type 2 diabetic patients. Regarding glibenclamide, our findings of higher serum concentration of Zn compared to the untreated group is in line with those obtained in a previous study on streptozotocin-induced diabetic rats (Shawky et al., 2018). In the present study, the increment in the serum Zn level in the treated groups may be related to the improvement in the glycaemic parameters, including FSG. A significant decrease in FSG might result in a decrease in Zn urinary loss and an increase in its active transport to tubular cells (Praveeena S., 2013). However, the glibenclamide treated group, unlike its metformin counterpart, did not show significant low FSG levels compared to the newly diagnosed group. In contrast, patients treated with glibenclamide showed comparable insulin levels to the control. Zn is important for synthesis and release of insulin, and its shortage appears to affect insulin release (Lobo et al., 2010). Therefore, the ability of glibenclamide to increase Zn levels could be related to its role in stimulation of insulin release from the pancreatic β-cells.

In the current study, the use of glibenclamide resulted in an increase in serum magnesium to a level comparable to the control and significantly higher than the newly diagnosed group. By contrast, metformin did not significantly change serum Mg levels, leading to significantly lower levels than the control. Our results are in accordance with the findings of previous studies (Chakraborty et al., 2011, Wahlen et al., 2017). Even though the glibenclamide treated group in the present study had worse glycaemic control, they showed better serum Mg levels than the patients treated with metformin. This outcome can be attributed to the ability of glibenclamide to increase insulin levels. This is in agreement with previous studies showing that insulin has an essential role in the renal reabsorption of Mg (Kurstjens et al., 2017) by stimulating the renal Mg channel TRPM6 (Nair et al., 2012). In contrast to our findings, Peters et al. (2013) reported lower serum magnesium concentrations in patients receiving metformin monotherapy or in combination with a sulfonylurea compared to those on diet alone.

It is necessary to emphasise that this study has several limitations due to restricted funds. Glycated haemoglobin (HbA1c) and intracellular magnesium were not measured. Moreover, collection of samples occurred at a single point, and no follow-up data is available.

Conclusion

The present study demonstrated an elevation in serum Cu and a reduction in serum Zn and Mg in T2DM. It also showed that metformin treatment is associated with decreased serum Cu and increased serum Zn. In contrast, glibenclamide treated patients revealed improvement in serum levels of Mg and Zn.

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