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# Effect of zinc as an add on to metformin therapy on serum lipid profile and uric acid in type 2 diabetes mellitus patients

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## ABSTRACT

Type 2 diabetes mellitus is a slowly progressive disease that may start insidiously and eventually results in secondary complications. The level of zinc in type 2 diabetic patients is significantly lower than in non-diabetics patients. Moreover, high zinc losses in urine further reduce serum zinc in all types of diabetes. This study aims to evaluate the effect of zinc supplementation, as an add-on to metformin therapy, on serum lipid profile and uric acid in type 2 diabetic patients versus metformin therapy alone. This is a case-control study that was conducted at the Diabetes and Endocrinology civil clinics and Diabetes Centre in Mosul, Iraq, from October 17th, 2020 to March 1st, 2021. The study included 67 type 2 diabetic patients. Metformin was provided to 32 patients (15 females and 17 males) as a control group. The other, interventional, group (n = 35; 16 females and 19 males) received metformin and zinc supplement. The serum lipid profile and uric acid level of the enrolled patients were measured at the baseline of the study and after two months, and the results of the two groups were compared. The results confirmed that zinc use significantly lowered total cholesterol, triglycerides, low-density lipoprotein, and Castelli's risk index I of atherogenicity and raised high-density lipoprotein level but did not affect very low-density lipoprotein. No changes were observed in serum lipid profile and uric acid levels in the metformin alone group. Zinc levels were substantially increased and reversed to normal in the zinc plus metformin case group in comparison to the metformin alone group. The study concluded that in type 2 diabetes mellitus patients, measuring zinc levels and compensating for the deficiency by zinc supplementation is recommended. This might carry a beneficial effect on serum lipid profile and uric acid levels, and hence diabetic outcomes.

**KEYWORDS:** diabetes mellitus, zinc, lipid profile, uric acid.

## INTRODUCTION

Type 2 diabetes mellitus (T2DM) has increased in prevalence and complications over the last two decades, with an increase in atherogenicity due to impairment in lipid profiles and high blood pressure. Zinc supplementation in T2DM reduces low-density lipoprotein (LDL-C), total cholesterol (TC), and triglyceride (TG) levels while raising high-density lipoprotein (HDL-C) [1]. In a meta-analysis of zinc usage in T2DM diabetes, researchers reported that zinc has a beneficial impact on nephropathy and neuropathy because it lowers TG and TC, eliminating macro and micro-vascular complications. Ranasinghe *et al*. concluded that zinc reduces TC, TG, and LDL-C in diabetic patients, and hence atherogenicity [2], while Khazdous *et al*., found a positive effect on VLDL-C, TC, and TG in their meta-analysis [3].

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In borderline cases of hyperglycemia, measuring zinc and uric acid aids in the early detection of diabetes, early recognition of complications, and improving the prognosis. Regarding uric acid, hyperuricemia can be caused by diabetes alone [4], and insulin resistance is linked to hyperuricemia [5]. Manideep *et al*. reported that uric acid has a protective role and it may act as an antioxidant (AO) during free radical (FR) formation [4]. Zinc, moreover, has an important role as a supporter to maintain the action of superoxide dismutase and metallothioneins, which are important for zinc and copper homeostasis, and protection against oxidative stress in islet cells and serum lipids [6]. This study was conducted to assess the effect of zinc supplementation, as an add on to metformin therapy, on serum lipid profile and uric acid level in T2DM patients.

## PATIENTS AND METHODS

A total of 67 patients with T2DM were enrolled in this study; 31 females and 36 males. They all live in Mosul city, Iraq, and came from a variety of socioeconomic backgrounds.

The patients were allocated into two groups; the first group (n = 35; 16 females and 19 males), was given metformin at a dose of 1000-2250 mg/day, in two-three divided doses after meals plus a single dose of 50 mg zinc gluconate every other day. The second group (n = 32; 15 females and 17 males) was treated with metformin alone at a dose of 1000-2250 mg/day. All the patients were instructed about how to improve their diet and to increase their physical activity by thirty minutes of brisk walking five days a week.

The patient's ages ranged from 31 to 64 years. The mean age ± standard deviation (SD) was 47.50 ± 8.95, and the mean body mass index (BMI) ± SD was 30.37 ± 3.19 for group one. Group two had a mean age of 51.10 ± 8.96 years and a mean BMI of 30.40 ± 3.46. All the patients were interviewed about age, tobacco smoking, duration of illness, presence of acute and chronic illnesses, and medication use.

From October 17$^{th}$, 2020 to March 1$^{st}$, 2021, a case-control study was conducted at the Diabetes and Endocrinology civil clinics and Diabetic Centres in Mosul, Iraq. At the beginning of the study, all the enrolled patients were tested for zinc levels, and they were then distributed into groups based on their zinc levels. Those who had little to low normal zinc levels were classified as interventional cases (group I), while the others were included in the control group (group II).

### Inclusion

Patients with T2DM who are not taking any drugs other than metformin were all included. They had to be on metformin for at least six months. Patients with other diseases, alcoholics and smokers, lactating or pregnant women, and patients on any drugs other than metformin were excluded from the study.

Total cholesterol and TG were calculated by the colorimetric method using spectrometry according to Allain and Fossati and Prencipe, respectively [7, 8]. The HDL-C was calculated using the precipitation method [9], while LDL-C was calculated using the following formula:

LDL-C level = TC minus HDL-C minus TG/5 [10].

VLDL-C was calculated via Friedewald equation:

VLDL-C level = TG/5 [10].

Castelli's risk index (CRI-1) was calculated using the following equation:

CRI-1 = TC/HDL-C [11].

The levels of uric acid in the blood were determined using the spectrometry process [12]. The principle of the assay is based on the conversion of uric acid to allantoin, carbon dioxide, and hydrogen peroxide by uricase enzyme with subsequent reaction of peroxide with chromogen resulting in colored complex formation which was quantified by a spectrometer. Spectrometry was used to determine the amount of zinc in the sample [13]. A reagent kit for determining zinc levels in serum was used (LTA S. r. l., Italy). The reagent has chromogen, which reacts with zinc to make a colored compound; the strength of color is dependent on the amount of zinc in a sample, and standard serum zinc concentration is 70-115 g/dl. The zinc level was measured using the formula as follows:

Serum zinc concentration = Specimen absorbance/ Standard absorbance * 200 μg/dl.

### Statistical analysis

The data obtained in the laboratory were categorized and coded using Microsoft Excel 2010. The statistical software Minitab version 18 was used to describe and analyze the data. The mean and SD were used as descriptive statistics for each measurable variable.

An independent T-test of two means was used to compare the case and control groups, while a paired T-test of two means was used to compare the pre and post-data for each group. A *p* value of ≤0.05 was used to indicate a significant difference.

## RESULTS

The lipid profile, uric acid, and serum zinc data at baseline of the case group (group I, n = 35) and the control group (group II, n = 32) are given in Table 1.

After two months of zinc administration in group I, there was a significant decrease in TC, TG, LDL-C, and CRI-1, with a significant rise in HDL-C and serum zinc level, but no significant difference in VLDL-C and the uric acid level was observed (Table 2).

In control group II, the criteria under investigation showed no major changes (Table 3).

Comparing the two groups at the end of the study, there was a significant difference between the two groups in terms of TC, LDL-C, HDL-C, CRI-1, and serum zinc level. There was no significant difference in uric acid levels and VLDL-C (Table 4).

## DISCUSSION

The difference between the two groups in terms of TC was highly significant (*p*-value = 0.008) after two months of using zinc supplement, indicating that zinc and metformin are superior to metformin alone in diabetes. Jayawardena *et al*. meta-analysis of zinc effect on diabetes reported that zinc significantly decreased TC in T2DM patients [14], which agrees with our findings. The findings for TG indicate that zinc and metformin are both beneficial in reducing TG levels in diabetes. Following our findings, Afkhami-Ardkani *et al*. found that zinc has a positive impact on TG levels [15].

The findings for HDL-C mean that zinc plus metformin are much superior to metformin alone in terms of HDL-C levels. A beneficial effect of zinc on HDL-C was reported in a meta-analysis by Asbaghi *et al.* [16] and in Khan *et al*. report, which revealed a significant increase of HDL-C

**Table 1.** Comparison of serum zinc level, lipid profile, serum uric acid level among the two groups at the start of the study.

| **Lipid profile parameters** | **Group I [n = 35] Mean ± SD** | **Group II [n = 32] Mean ± SD** | ***p*-value*** |
|---|---|---|---|
| S. zinc (mg/dl) | 68.6 ± 11.2 | 74.0 ± 15.7 | 0.107 |
| Cholesterol (mg/dl) | 196.9 ± 34.0 | 200.2 ± 48.4 | 0.750 |
| TG (mg/dl) | 160.8 ± 60.1 | 132.3 ± 59.4 | 0.056 |
| HDL-C (mg/dl) | 43.00 ± 5.17 | 42.20 ± 5.32 | 0.529 |
| LDL-C (mg/dl) | 125.1 ± 30.5 | 130.4 ± 46.6 | 0.581 |
| VLDL-C (mg/dl) | 31.4 ± 12.5 | 26.9 ± 11.9 | 0.140 |
| TC/HDL-C (CRI-1)** | 4.65 ± 1.28 | 4.83 ± 1.50 | 0.599 |
| S. uric acid (mmol/l) | 4.85 ± 1.46 | 4.40 ± 1.18 | 0.168 |

* Independent T-test of two means was used.

** TC/HDL-C (Castelli's risk index I)(CRI-1).

**Table 2.** The effect of zinc administration on serum zinc level, lipid profile, and uric acid level in group I.

| Parameters | Group I [n = 35] | | *p*-value* |
|---|---|---|---|
| | Baseline Mean ± SD | After two months zinc Mean ± SD | |
| S. zinc mg/dl | 68.6 ± 11.2 | 85.4 ± 14.9 | **0.001** |
| Cholesterol (mg/dl) | 196.9 ± 34.0 | 176.5 ± 28.9 | **0.001** |
| TG (mg/dl) | 160.8 ± 60.1 | 147.1 ± 53.7 | **0.042** |
| HDL-C (mg/dl) | 43.00 ± 5.17 | 45.66 ± 4.38 | **0.002** |
| LDL-C (mg/dl) | 125.1 ± 30.5 | 102.5 ± 29.2 | **0.001** |
| VLDL-C (mg/dl) | 31.4 ± 12.5 | 29.9 ± 10.9 | 0.299 |
| Chol/HDL-C (CRI-1) | 4.65 ± 1.28 | 3.87 ± 0.83 | **0.001** |
| S. uric acid (mmol/l) | 4.85 ± 1.46 | 4.56 ± 1.09 | 0.197 |

* Paired T-test of two means was used.

**Table 3.** The variations in serum zinc level, lipid profile, and uric acid level after 2 months in group II.

| Parameters | Group II [n = 32] | | *p*-value* |
|---|---|---|---|
| | Baseline Mean ± SD | After two months Mean ± SD | |
| S. zinc (mg/dl) | 74.0 ± 15.7 | 73.8 ± 14.4 | 0.927 |
| Cholesterol (mg/dl) | 200.2 ± 48.4 | 198.3 ± 36.1 | 0.815 |
| TG (mg/dl) | 132.3 ± 59.4 | 140.9 ± 54.2 | 0.434 |
| HDL-C (mg/dl) | 42.20 ± 5.32 | 42.37 ± 5.76 | 0.881 |
| LDL-C (mg/dl) | 130.4 ± 46.6 | 127.0 ± 36.8 | 0.678 |
| VLDL-C (mg/dl) | 26.9 ± 11.9 | 28.3 ± 10.8 | 0.525 |
| Chol/HDL-C (CRI-1) | 4.83 ± 1.50 | 4.70 ± 1.17 | 0.679 |
| S. uric acid (mmol/l) | 4.40 ± 1.18 | 4.48 ± 0.89 | 0.676 |

* Paired T-test of two means was used.

by zinc administration [17]. Zinc may play a role in plasma HDL-C regulation according to Lodovici *et al*. that found a positive association between HDL-C, antioxidants, and malondialdehyde in diabetes mellitus patients [18]. These results indicate that HDL-C and antioxidants are involved in the compensatory response to oxidative stress induced by elevated glucose levels [6].

Zinc and metformin are much superior to metformin alone in lowering LDL-C levels. Asbaghi *et al*. analysis agree with our findings in terms of LDL-C [16] while Khan *et al.,* found contradictory findings [17]. In terms of CRI-1 in diabetes, zinc and metformin are often much superior to metformin alone. In patients with a high risk of cardiovascular disease, Ma *et al*.

**Table 4.** Comparison of serum zinc level, lipid profile, and serum uric acid level among the two groups after 2 months follow-up.

| **Lipid profile parameters** | **Group I [n = 35] Mean ± SD** | **Group II [n = 32] Mean ± SD** | ***p*-value*** |
|---|---|---|---|
| S. zinc (mg/dl) | 85.4 ± 14.9 | 73.8 ± 14.4 | **0.002** |
| Cholesterol (mg/dl) | 176.5 ± 28.9 | 198.3 ± 36.1 | **0.008** |
| TG (mg/dl) | 147.1 ± 53.7 | 140.9 ± 54.2 | 0.643 |
| HDL-C (mg/dl) | 45.66 ± 4.38 | 42.37 ± 5.76 | **0.010** |
| LDL-C (mg/dl) | 102.5 ± 29.2 | 127.0 ± 36.8 | **0.004** |
| VLDL-C (mg/dl) | 29.9 ± 10.9 | 28.3 ± 10.8 | 0.533 |
| Chol/HDL-C | 3.87 ± 0.83 | 4.70 ± 1.17 | **0.001** |
| S. uric acid (mmol/l) | 4.56 ± 1.09 | 4.48 ± 0.89 | 0.720 |

***** Independent T-test of two means was used.

found a negative association between zinc levels and the risk index ratio [19]. This is consistent with our findings. Moreover, Sasikala and Goswami in their study found that the use of Castelli's index was a better guide for screening and early recognition of cardiometabolic risk [20].

Zinc can improve lipid profile either by improving glycaemic regulation or by lowering the sensitivity of lipoprotein and other important proteins to oxidation by lowering the amount of harmful FR [6, 21, 22]; hence, zinc participates in potentiating intrinsic antioxidant mechanisms and reducing the complication of FR formation which helps in decreasing lipid peroxidation [6]. This may be linked to the ability of zinc to reduce glycated hemoglobin by 0.6% and when mixed with metformin, which lowers HbA1c by 0.6% as well, both can alleviate the complication of diabetes by 32% and lower mortality by 42% [14].

El-Ashmony *et al*. reported that zinc is a beneficial supplement in terms of lipid profile in diabetic patients, lowering TC, TG, and LDL-C while substantially increasing HDL-C in the zinc group compared to the placebo group [6], which is in agreement with our findings. Moreover, Al-Maroof and Al-Sharbatti reported that zinc is advantageous for diabetes by lowering TC, TG, and LDL-C, as well as atherogenicity, morbidity, and mortality [23]. Conversely, Partida-Hernandez *et al*. found no effect on LDL-C and a positive effect on lowering TC, TG, and increasing HDL-C [24].

In their meta-analysis, Pompano and Boy showed that various zinc doses and durations have different effects and benefits on a variety of factors. They revealed that low doses are better than large doses for avoiding unfavourable side effects regardless of zinc therapy duration; this is because high doses can cause zinc unfavourable effects; another advantage of the low dose is better absorption, as large doses can result in saturation of the natural system. As a result, they hypothesized that low doses are safe and can be administered for longer periods. They found that low dose (≤25 mg/day) benefits TC, TG, and LDL-C, and short duration (<12 weeks) showed an improvement of TG. On the contrary, a high dose (≥25 mg/day) benefits TG only while a long-duration treatment with zinc (>12 weeks) showed improvement of TC, TG, and LDL-C [25]. In our study, the low dose of zinc administration for a short duration proved to be beneficial in terms of TC, TG, LDL-C as well as HDL-C and CRI-1.

Concerning uric acid levels, this study revealed that zinc co-administration with metformin carries no significant effects on its level. Uric acid acts as an AO during the formation of FR, according to Manideep *et al.* [4]. Many researchers found varying levels of uric acid; while some found it

to be elevated in T2DM, others found it to be low. Manideep *et al*. and Hisalkar *et al*. in their trials to prevent or delay diabetes onset found that uric acid, as an antioxidant, was reduced in diabetic patients [4, 21]. In contrast, Matough *et al.* reported that uric acid was elevated in T2DM [4]. A report by Zhang *et al.* demonstrates an inverse relationship between zinc and uric acid in an assessment of the results between dietary zinc and hyperuricemia [26]. In reality, zinc reduces uric acid levels, as shown by the current study findings, but this reduction was not significant.

To the best of our knowledge, this is the first study defining the role of zinc in modulating uric acid level and lipid profile in diabetic patients on metformin therapy. Although some suggest genetic factors are involved, low zinc levels are caused by poor absorption and/or increased urinary loss as a result of changed renal function [27]. Low zinc levels can also be caused by hyperglycemia, which causes glucose loss in the urine and zinc deficiency [6]. Al Maroof and Al-Sharbatti reported that diabetic patients had significantly lower mean serum zinc levels than healthy controls (68.9+/-11.9 mg/dl in T2DM diabetic patients versus 83.4+/-12.5 mg/dl in healthy controls), and this is in agreement with our finding. According to Al Maroof and Al Sharbatti, zinc supplementation at a dose of 30 mg/day for 12 weeks significantly increased serum zinc levels [23].

## CONCLUSION

In T2DM patients, measuring zinc levels and restoring them to normal levels by providing zinc supplementation is recommended. This might carry a beneficial effect on serum lipid profile and zinc levels.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

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