Effect of high-dose zinc supplementation with oral hypoglycemic agents on glycemic control and inflammation in type-2 diabetic nephropathy patients

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Abstract

Objective: The study aims to evaluate the effect of zinc sulfate on markers of glycemic control, lipid profile and inflammation in type-2 diabetes with microalbuminuria patients. **Materials and Methods:** Type-2 diabetes with microalbuminuria patients on oral hypoglycemic agents (OHA) and angiotensin converting enzyme (ACE) inhibitors were selected and divided into 2 groups: One group ($n = 27$) continued with OHA alone, second group ($n = 27$) was on OHA and in addition 50 mg elemental zinc as zinc sulphate supplementation for 12 weeks. Fasting, post-prandial blood glucose, glycosylated hemoglobin, lipid profiles, inflammatory marker hs-CRP and urine microalbumin were measured. **Results:** There were no significant differences in biochemical status among groups at baseline. After receiving zinc, the mean fasting blood glucose (FBS), post-prandial blood glucose (PPBS) and glycosylated hemoglobin (HbA1c) were decreased significantly ($P = 0.0001$). Significant decrease was observed in TG ($P = 0.002$) and VLDL-cholesterol ($P = 0.002$), whereas there was no significant decrease in TC and LDL-cholesterol. The high-density lipoprotein (HDL) cholesterol was significantly ($P = 0.0001$) increased from baseline. Zinc supplementation had significant effects in decreasing serum hs-CRP from 10.51 ± 1.68 mg/L to 7.75 ± 1.56 mg/L ($P = 0.0001$) and microalbumin level from 146.87 ± 30.83 mg/day to 80.70 ± 33.99 mg/day ($P = 0.0001$). There were no significant changes in the levels of all these parameters in OHA group. **Conclusion:** Our results conclude that supplementation of zinc improved the effectiveness of OHA and may be beneficial in decreasing blood glucose, TG, urinary albumin excretion and inflammation in diabetic nephropathy patients and thus reducing the risk of complications.

Key words: Glycemic control, inflammation, microalbuminuria, type-2 diabetes, zinc sulphate

INTRODUCTION

Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) worldwide. Nearly 30% of chronic renal failures in India are due to diabetic nephropathy.[1] The earliest clinical evidence of nephropathy is the appearance of low but abnormal levels (>30 mg/day) of albumin in the urine, referred to as microalbuminuria (MAU). Without specific interventions, 20% to 40% of type 2 diabetic patients with microalbuminuria progress to overt nephropathy.[2]

Microalbuminuria is also associated with endothelial damage.[3,4] C-reactive protein (CRP) is a very sensitive marker of low-grade systemic inflammation, and it has been suggested that this acute phase protein impairs

vascular endothelial function. Studies have shown positive association between CRP and nephropathy as measured by albuminuria.[5-8] Both endothelial dysfunction and inflammation are involved in the pathogenesis of MAU and poor glycemic control was associated with increase in markers of endothelial dysfunction and inflammatory activity.[6] A dyslipidemic lipid profile, namely low-high density lipoprotein and elevated low-density lipoprotein/ triglyceride levels, has also been shown to be related to microalbuminuria.[9]

Zinc is an essential mineral that is required for various cellular functions. Zinc is considered important mainly because it plays a major role in the stabilization of insulin hexamers and the pancreatic storage of the hormone[10] and it may play a critical role as a potent anti-oxidant and anti-inflammatory agent.[11,12] Zinc may improve glycemia, and a restored zinc status in patients with type-2 diabetes may counteract the deleterious effects of oxidative stress, helping to prevent complications associated with diabetes.[13]

Previous studies indicated that marginal zinc deficiency is more prevalent among diabetic adults, compared with the normal adult population.[14,15] The above-mentioned considerations, seem reasonable that zinc might be used to treat or prevent diabetic nephropathy. It is not known whether zinc supplements given together with oral hypoglycaemic agents (OHA) would increase the efficacy of the drugs and retard inflammation. The present study was designed to determine the effect of zinc supplementation with OHA treatment in type-2 diabetes with microalbuminuria patients in North Indian population.

MATERIALS AND METHODS

Study population
The study has been carried out in type-2 diabetic patients attending the outpatient Department of Medicine, at C.S.M. Medical University, Lucknow (Erstwhile King George's Medical College), in North Indian patients had been screened for microalbuminuria.

Diabetic patients with nephropathy, in the age group of 40-69 years, with fixed OHA dosage and receiving angiotensin-converting enzyme inhibitors or angiotensin receptor blockers for microalbuminuria recruited for supplementation.

Patients with concurrent acute illness, including infectious disease, malignancy, and active immunological diseases, medical history of clinical cardiovascular disease, tuberculosis, pregnancy, lactation, using corticosteroids or other medicines such as statins, or vitamins, or mineral supplements in the past 3 months, severe uncontrolled hypertension (>160/100 mmHg) or renal insufficiency (serum creatinine > 1.5 mg/dL), and smoking history were excluded from the study.

A detailed interview by using a structured questionnaire was documented. Furthermore, their dietary intakes were assessed using a 24-hour dietary recall questionnaire at their entry into the study. Body mass index was calculated as the weight in kilograms per square of height in meters, and blood pressure measured with the person in the sitting position after a 5-min rest.

Study design and supplementation
A total of 54 type-2 diabetic patients with microalbuminurea were randomly assigned to 2 treatment groups (OHA-alone: 27, OHA plus zinc: 27). Out of these, a total of 44 patients (OHA–alone: 21, OHA plus zinc: 23) completed the study. Each capsule 50 mg Zn (as zinc sulphate) and OHA consisted of drugs only. One capsule of 50 mg zinc per day was taken before breakfast with plenty of water. Zinc capsules were supplied by Shalaks Pharmaceuticals Pvt. Ltd (New Delhi, India). All patients were examined carefully and depending on the treatment groups, blood pressure, drug complications, such as nausea, vomiting, abdominal pain, diarrhea, constipation, reduction of appetite were checked after 3 weeks. Four patients in the OHA plus zinc group (2 patients needed change of drugs on statins, and 2 other patients started with insulin) and 6 in the OHA-alone group lost to follow-up for poor compliance. At the end of 12 weeks all the indices were checked as before the beginning of the test and the drug complications were asked as well.

Ethical considerations
The study has been approved by the Institutional Ethics Committee of CSM Medical University, Lucknow, UP, India. Informed consent was obtained from each subject before the start of the study.

Blood collection and biochemical assay
After an overnight, fasting and post-prandial (2 hours after lunch) blood samples were obtained. Samples were collected in EDTA and sterile tubes. Serum samples were separated by centrifugation at 3000 r/min during a period of 15 minutes. For lipid and lipoprotein determinations, serum was kept at 4°C until its processing within 48 hours. A serum aliquot was stored at −80°C for measurement of hs-CRP. The same procedure was followed at an interval of 3 months. The glycosylated hemoglobin (HbA1C) was estimated in ethylenediamine tetra-acetic acid anticoagulated whole blood with preparation of hemolysate sample. The HbA1C concentration was measured by ion exchange chromatography. Fasting and post-prandial blood glucose levels were measured

by an automated enzymatic method. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, serum triglyceride, and low-density lipoprotein (LDL) cholesterol (LDL-C) were estimated by standard methods. Marker of inflammation high-sensitivity C-reactive protein (hs-CRP) was measured by particle-enhanced immunologic agglutination (Roche Diagnostics, GmbH, Mannheim). The procedure had a sensitivity of 0.2 mg/L.

Chemicals and reagents
All chemicals and reagents of excellent quality obtained from Randox Laboratories Ltd, Crumlin Antrim, United Kingdom, have been used for blood glucose and lipid profile estimations.

Urinary albumin excretion assay: A 24-hour quantitative determination of microalbumin in urine by turbidimetric immunoassay was done, which is based on antigen-antibody reaction in measurement by the end point method (Erba Diagnostics Mannheim GmbH, Mallaustrasse Mannheim/Germany).

Statistical analysis
The data collected was entered in Microsoft Excel program and was checked for any inconsistency. A one-sample Kolmogorov-Smirnov test was used to investigate whether the variables were normally distributed. The unpaired t-test was used to investigate the differences at the baseline values among 2 groups. The paired t-test was used to compare changes from baseline to follow-up. The statistical significance was accepted at a probability level of 0.05. Analyses were performed by using SPSS software package (WINDOWS version 15.0: SPSS Inc., Chicago, IL, USA).

RESULTS

The groups were similar with respect to age, duration of diabetes, BMI, daily calorie and nutrient intake. [Table 1] This study shows that lower dietary zinc intakes and glucose intolerance was also more prevalent in diabetic nephropathy in north India. The treatment, diet and physical activity of the patients remained unchanged during the course of study. Almost all patients were treated with metformin and/or sulphonylurea. None of the patients took insulin.

There were no significant differences in Fbs, PPbs, HbA1C, lipid profile and serum hs-CRP levels between the 2 groups before the treatment at baseline [Table 2].

As shown in Table 3, fasting blood sugar, post-prandial blood sugar and HbA1C were significantly ($P < 0.0001$) decreased from baseline in OHA plus zinc group. The level

Table 1: Demographic, anthropometric and total dietary intake of diabetic nephropathy patients

Baseline characteristics	OHA plus zinc group (n=27)	OHA-alone group (n=27)
Age (years)	56.3±6.6	56.0±8.6
Diabetes mellitus duration (years)	10.04±2.90	8.57±4.33
Body mass index (kg/m²)	26.80±3.02	26.15±3.01
Systolic blood pressure (mm Hg)	137.48±6.86	140.76±7.22
Diastolic blood pressure (mmHg)	86.78±4.34	82.95±4.32
Energy (kcal)	1690.80±130.20	1618.24±146.46
Carbohydrate (g)	254.20±11.90	240.24±14.12
Protein (g)	58.40±8.20	60.20±9.86
Fat (g)	42.38±4.10	40.82±6.62
Dietary zinc (mg/day)	5.22±1.60	4.88±0.94
Dietary copper (mg/day)	1.06±0.48	1.10±0.56

Data are means±SD, there were no significant differences between groups by t-test, OHA: Oral hypoglycemic agents

Table 2: Baseline biochemical parameters of diabetic nephropathy patients

Baseline parameters	OHA plus zinc group (n=27)	OHA-alone group (n=27)	P value
Fasting blood glucose (mg/dL)	147.09±39.76	147.43±28.94	0.98
Post-prandial glucose (mg/dL)	195.30±47.76	208.71±61.52	0.56
HbA1C (%)	8.35±0.87	8.45±0.92	0.99
Total cholesterol (mg/dL)	151.52±19.10	150.29±26.89	0.85
Triglycerides (mg/dL)	155.48±41.03	155.66±26.40	0.77
HDL-cholesterol (mg/dL)	30.04±6.41	30.50±8.74	0.93
LDL-cholesterol (mg/dL)	89.96±20.74	88.82±25.66	0.72
VLDL-cholesterol (mg/dL)	31.39±8.97	30.07±5.88	0.33
hs-CRP (mg/L)	10.51±1.68	10.48±2.71	0.67
Urine microalbumin (mg/day)	146.87±30.83	145.05±45.97	0.81

HbA1C: Glycosylated hemoglobin, HDL: High density lipoprotein, hs-CRP: High-sensitive C-reactive protein, LDL: Low-density lipoprotein, OHA: Oral hypoglycaemic agents, VLDL: Very-low density lipoprotein

of serum triglyceride was significantly ($P = 0.002$) decreased in OHA plus zinc group compared with OHA-alone group. There was no significant difference in the level of LDL and total cholesterol from baseline after supplementation. The HDL cholesterol was significantly ($P < 0.0001$) increased from baseline to 12 weeks.

Inflammatory marker hs-CRP was significantly ($P < 0.0001$) decreased from baseline (10.51 ± 1.68 mg/L) to after 12 weeks (7.75 ± 1.56 mg/L) zinc supplementation. Highly significant ($P < 0.0001$) decrease was observed in the level of urine microalbumin level in OHA plus zinc group.

DISCUSSION

Taking multivitamin along with oral hypo-glycemic agents

Table 3: Effect of zinc supplementation in type-2 diabetic nephropathy patients

Parameters	OHA plus zinc group (n=23)		P value	OHA-alone group (n=21)		P value
	Pretrial	Posttrial		Pretrial	Posttrial	
Fasting blood sugar (mg/dL)	147.09±39.76	113.74±23.38	<0.0001*	147.43±28.94	154.95±23.38	0.33
Post-prandial blood sugar (mg/dL)	195.30±47.76	147.61±27.52	<0.0001*	208.71±61.52	218.05±52.15	0.93
HbA1C (%)	8.35±0.87	6.91±0.67	<0.0001*	8.45±0.92	8.61±0.92	0.04*
Total cholesterol (mg/dL)	151.52±19.10	139.74±17.32	0.06	150.29±26.89	157.10±26.50	0.53
Triglycerides (mg/dL)	155.48±41.03	111.61±25.37	0.002*	155.66±26.40	176.86±60.01	0.13
HDL-cholesterol (mg/dL)	30.04±6.41	40.83±6.66	<0.0001*	30.50±8.74	30.62±6.98	0.98
LDL-cholesterol (mg/dL)	89.96±20.74	77.96±14.53	0.06	88.82±25.66	91.19±21.05	0.64
VLDL-cholesterol (mg/dL)	31.39±8.97	22.04±5.02	0.002*	30.07±5.88	35.19±11.86	0.15
hs-CRP (mg/L)	10.51±1.68	7.75±1.56	<0.0001*	10.48±2.71	10.57±3.00	0.64
Urine microalbumin (mg/day)	146.87±30.83	80.70±33.99	<0.0001*	145.05±45.97	157.43±49.51	0.02*

HbA1C: Glycosylated hemoglobin, HDL: High-density lipoprotein, hs-CRP: High-sensitivity C-reactive protein, LDL: Low-density lipoprotein, OHA: Oral hypoglycemic agents, VLDL: Very-low density lipoprotein, *Significant at <0.05 level

was a very common practice in diabetic patients with complications, but recently the role of zinc has been found to be effective in lowering of blood sugar level and inflammation in diabetic nephropathy.

The present study indicates that microalbuminuria patients were not under a tight glycemic control. More significant effects occurred in levels of FBS, PPBS and HbA1C after 12 weeks, which may be related to the higher dosage of zinc sulphate. The present study is consistent with the previous reports.[16,17]

A study by Anne-Marie of 56 diabetic patients treated with 30 mg zinc gluconate showed that HbA1c decreased from 8.9 ± 0.4 to 7.7 ± 0.3% following 6 months of zinc supplementation but the decrease was not significant.[18] We have observed highly significant changes in glycemic control due to megadose of zinc in our study.

Zinc treatment was well tolerated and significantly reduced the triglyceride concentrations ($P = 0.002$) and VLDL cholesterol ($P = 0.002$), whereas it significantly increased the levels of HDL cholesterol ($P = 0.0001$). Therefore, the present study confirmed the lipid-lowering effects of zinc in humans.[16,17,19] Some investigations indicated that a zinc-enriched diet has beneficial effects on basal and post-prandial glycemia, the content of cholesterol and triglycerides.[20]

Garber and Karlsson showed that the treatment of dyslipidemia in diabetes must be focused on several targets involving glycemic control and reduction of LDL levels.[21] The results of the present study are agreeable with these suggestions. There is evidence suggesting that zinc can act as an endogenous protective factor against atherosclerosis by inhibiting the oxidation of LDL in the presence of transition metals.[22]

Furthermore, the level of inflammatory marker hs-CRP was significantly decreased ($P = 0.0001$) in type-2 diabetic microalbuminuria patients. Therefore, the present study is in agreement with the previous report of Bao et al., which had shown reduction of inflammation in elderly subjects with 45 mg zinc per day supplementation for 6 months.[23] A previous study used 45 mg zinc per day as supplementation in elderly individuals for 1 year. This dose of zinc was effective in correcting immune dysfunction.[24]

The PPAR-α and -γ of nuclear receptors, the mediators for lipoprotein metabolism, inflammation, and glucose homeostasis, were shown to play an important protective role in the development and progression of atherosclerosis.[25] The mechanisms by which zinc has atheroprotective function may be due to its anti-inflammatory effect by down-regulation of atherosclerosis-related NF-κB activation via negative cross-talk in the nuclear DNA binding level.[26] The activation of PPAR-α and -γ and the down-regulation of inflammatory cytokines and endothelial cell adhesion molecules in endothelial cells were reported to be zinc-dependent.[27]

The present study showed marked reduction of urinary albumin excretion, a marker for renal function. Findings of the present study are consistent with recently published studies, in which the urinary albumin excretion was seen to be decreased in diabetic patients who had received zinc.[28,29]

In the present study, we have shown that zinc supplementation improves the glycemic and lipid profile of diabetic patients with microalbuminuria. However, we do not know if positive effects on insulin capacity could play critical roles in the underlying biological mechanisms. Serum zinc and urinary zinc measurements were not taken, which limits the explanatory power of our study in this regard; nor did we perform insulin levels, which could supply information about the patients' insulin capacity.

Therefore, we recommend to include insulin and zinc estimation in future studies.

Since the complications of diabetes may be mediated, at least in part, through oxidative stress, which potentially affect the heart, vascular system, kidney, retina and peripheral nerves; zinc plays a key role in the cellular anti-oxidative defense.[30] This was confirmed in the current study; it seems that zinc is a proper mineral supplement in diabetic patients owing to its deficiency.

CONCLUSION

The supplementation improved the effectiveness of OHA in combination with zinc in diabetic nephropathy patients. The result of the present study showed that zinc supplementation successfully reverted inflammation and improve the renal function in diabetic patients. Zinc may have a protective effect in atherosclerosis because of its anti-inflammatory and anti-oxidant functions.

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