ORIGINAL ARTICLE

Comparison of Two Supplementary Zinc Doses on Lipid Peroxidation in Diabetic Patients

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ABSTRACT

OBJECTIVE: The present study was performed to compare the antioxidant effect of two different doses of zinc on lipid oxidation in type 2 diabetic patients.

MATERIALS AND METHODS: This double-blind, randomized controlled trial was conducted on 60 type 2 diabetic patients in Yazd Diabetes Research Center in 2008. The subjects were randomly allocated to receive Zinc sulfate (Iran, Alhavi) either 220 mg (containing 50 mg zinc) or 110 mg (containing 25 mg zinc) daily for two months.

RESULTS: In the present study it was found that serum levels of zinc at the end of trial differed significantly after 50 mg/day zinc supplementation ($P = 0.002$), but this difference was not observed with dose of 25 mg/day zinc administration. There were no significant differences in the quantitative parameters of serum lipid oxidation after either of two doses of Zinc Sulfate ($P > 0.05$).

CONCLUSION: This study showed zinc supplementation with 50 mg daily for two months could increase serum level of zinc significantly, but we did not observed any change in susceptibility of serum lipid oxidation by 25 or 50 mg zinc supplementation in diabetic patients.

KEYWORDS: Type 2 Diabetes, Zinc Supplementation, Lipid Peroxidation

INTRODUCTION

Diabetes mellitus is one of the most common problems caused by a combination of insulin resistance and impaired insulin secretion by pancreatic *B* cells (1). There has been currently great interest in the potential contribution of increased oxidative stress to the development of diabetes complications (2). An increase in oxidative stress may occur due to an increase in free radical production (3). Several mechanisms seem to be involved in the pathogenesis of the oxidative stress, which include glucose autoxidation, protein glycation and formation of advanced glycation endproducts, and the polyol pathway (4).

Since oxidative stress may be a contributing factor in diabetes complications, supplementation with antioxidants could be of interest, by prevention or at least delay in development of vascular complications (5).

Zinc is a micronutrient with known antioxidant properties (6) but its antioxidant

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mechanism isnot known (5,6). Zinc salts with low toxic profiles have been shown to have anti-diabetic effects in diabetic experimental mice (7). Also, zinc has been found to have in vitro insulinomimetic activity and in vivo blood glucose lowering effect (8). It has been shown that patients with diabetes have lower serum levels of zinc (9). Faure et al. noted that correction of zinc deficiency in diabetic patients causes a reduction in lipid peroxidation (10).

The present study was performed to compare the antioxidant effect of two different doses of zinc on lipid oxidation in type 2 diabetic patients.

MATERIALS AND METHODS

This double-blind, randomized controlled trial was conducted on 60 type 2 diabetic patients, aged 40 to 60 years in Yazd Diabetes Research Center in 2008.

The volunteers who met the following inclusion criteria were enrolled in the study: having type 2 diabetes mellitus for more than 5 years (diagnosed according to ADA criteria) (11) with glycated hemoglobin (HbAlc) more than 7%, triglyceride (TG) less than 400 mg/dl and total cholesterol (TC) less than 250 mg/dl.

Exclusion criteria were: Cigarette smoking, current treatment with anti-oxidant drugs or other medication except oral hypoglycemic agent, diabetic complications, using vitamins or minerals supplements in the last two months, renal failure, ischemic heart disease, congestive heart failure, uncontrolled hypertension, history of stroke and chronic liver disease, pregnancy and lactation. Moreover, patients who had to change dosage or types of medications were excluded.

The University Ethics Committee approval was obtained prior to study enrollment. Informed consent was obtained in all cases. At the first visit, demographic and clinical information was documented.

In this study neither physical activity nor food planning was modified. The subjects were all given advice to unchanged dietary program and physical activity during the study. Adherence to pharmacological treatment and lifestyle intervention was assessed every month by personal interview and the subjects were asked about any side effects.

A total of 60 eligible subjects were enrolled and randomly allocated to receive Zinc sulfate (Iran, Alhavi) either 220 mg (containing 50 mg zinc) or 110 mg (containing 25 mg zinc) daily for two months. (The reason of selected dose was gastrointestinal problem in higher dose and the intervention of higher dose with other mineral elements like Fe, Ca, etc.)

Laboratory Methods: Blood samples were obtained before and after intervention. Serum separated from the clots after complete coagulation (l h in room temperature) by low speed centrifugation (15 min at 2000 g), and stored in -70°C refrigerator.

Serum glucose was measured by colorimetric method (GPO-PAP) with Photometer 5010 (Parsazmon kit, Iran). HbA1c was measured by DS5 analyzer and DS5 Pink Reagent kit.

Copper-induced serum lipid peroxidation was estimated in a 60-fold diluted serum in 20 mM phosphate buffer containing 720 uM sodium citrate, pH = 7.4. The lipid oxidation procedure was performed at 37°C and was initiated by addition of CuCl2 to give a final concentration of 60 uM. The kinetics of conjugated dienes formation were monitored by spectrophotometer (Perkin-Elmer UV.VIS Double beam spectrophotometer 505S) by measuring absorbance in a 1-cm quartz cuvette at 245 nm, every 10 min for 300 min. For plotting the kinetic curves of the accumulation of lipid peroxide products (change of absorbance at 245 nm versus time in min) Microsoft Excel software was used. A number of quantitative oxidation parameters including lag-time (the interval between the addition of CuCl2 to the serum and the beginning of extensive oxidation), maximal rate of oxidation (V -max), maximal amount of lipid peroxide products accumulation (OD-max), and the time needed to gain maximal rate of oxidation (T-max) were evaluated (18). Before processing the samples, method of serum lipid oxidation was optimized and an inter-individual coefficient of variations (Cv) of 6%

(for lag-time), 7.4% (for OD-max) and 7.5% (for V-max) were obtained. Zinc concentration was measured by atomic absorbance spectrophotometer (20AA model, the method of Varian Company). We dissolved 0.500 g zinc metal in a minimum volume of 6 M HCL, and then dilute to 1 L with 0.1 M HCL. To prepare the series of standards or zinc analysis, five concentrations were selected to set up a standard curve.

Units Normally a spectrophotometer is linear within the range of 0.05 to 1.0 absorbance. Standards should be prepared to give an absorbance within this range. A standard containing 0.5 ug/mL Zn will typically give an absorbance of about 0.15. The five following standard concentrations can be conveniently prepared from the stock solution: 0.5 ug/mL, 1.0 ug/mL, 1.5 ug/mL, 2.0 ug/mL, and 2.5 ug/mL.

Statistical analysis was performed using SPSS version 11.5, Chicago IL. To compare before and after metabolic responses Wilcoxon test and for comparing variable differences between two groups Mann Withney-test were used. Significance was considered to be $P < 0.05$. Data were presented as means ± SD.

RESULTS

In this study 60 type 2 diabetic patients who met the inclusion criteria were randomized to receive two different doses of zinc sulfate. Six subjects from the first group (110 mg/daily) dropped out because they were unwilling to continue the study and two patients had gastrointestinal side effects. In the second group (220 mg/daily), 5 patients stepped out due to having gastrointestinal side effects and three patients because of acute illness. Adherence to treatment was achieved for 22 subjects in either group.

Table 1 and 2 show the parameters before and after any trials. It was found in the present study that serum levels of zinc at the end of trial differed significantly after 50 mg/day zinc supplementation ($P = 0.002$), but this difference was not observed with dose of 25 mg/day zinc administration. (Presumably some patients forgot drug consumption because they

Table 1- Mean of quantitative serum oxidation parameters before and after zinc sulfate (110 mg/day) consumption

	Zinc Sulfate (110 mg/day) (n = 22)		
Variable	**Before**	**After**	**Wilcoxon-test P-value**
	Mean ± SD	**Mean ± SD**	
OD-max	0.33 ± 0.076	0.34 ± 0.081	0.55
T-max (Min)	139.1 ± 17.2	142.2 ± 21.2	0.48
V-max (OD/Min)	2.29 ± 0.74	2.27 ± 0.63	0.61
Lag-time (Min)	74.6 ± 11.4	74.7 ± 14.65	0.87
Serum Zinc (µg/dl)	130.3 ± 27.2	127.2 ± 23.3	0.48

Lag-time = the time needed (in min) for initiation of lipid oxidation products accumulation during the lipid oxidation course after addition of $CuCl_2$
OD-max = maximal amount of lipids peroxide products accumulation during the lipid oxidation course
V-max = maximal rate of oxidation during the lipid oxidation course
T-max = time needed (in min) to gain the maximal rate of lipid peroxide products accumulation during lipid oxidation

Table 2- Mean of quantitative serum oxidation parameters before and after zinc sulfate (220 mg/day) consumption

	Zinc Sulfate (220 mg/day) (n = 22)		
Variable	**Before**	**After**	**Wilcoxon-test P-value**
	Mean ± SD	**Mean ± SD**	
OD-max	0.33 ± 0.04	0.34 ± 0.07	0.48
T-max (Min)	130.9 ± 23.3	130.9 ± 21.2	1.00
V-max (OD/Min)	2.27 ± 0.36	2.23 ± 0.48	0.4
Lag-time (Min)	73.95 ± 18.1	69.13 ± 15.5	0.1
Serum Zinc (µg/dl)	140.14 ± 30.2	160.1 ± 30.3	0.002

Table 3- Mean of quantitative serum oxidation parameters in two groups at the end of the study

Variable	Zinc Sulfate (110 mg/day)	Zinc Sulfate (220 mg/day)	Mann Withney-test P-value
	Mean ± SD	Mean ± SD	
OD-max	0.34 ± 0.081	0.34 ± 0.07	0.618
T-max (Min)	142.2 ± 21.2	130.9 ± 21.2	0.055
V-max (OD/Min)	2.27 ± 0.63	2.23 ± 0.48	0.99
Lag-time (Min)	74.7 ± 14.65	69.13 ± 15.5	0.232
Serum Zinc (µg/dl)	127.2 ± 23.3	160.1 ± 30.3	0.00

P value < 0.05

consumed zinc sulfate every other day).

There were no significant differences in the quantitative parameters of serum lipid oxidation after either of two doses of Zinc Sulfate ($P > 0.05$).

When the differences of these laboratory levels between two groups were tested, no significant difference was found in Vmax, OD max, Tmax, Lag Time ($P > 0.05$) (Table 3).

DISCUSSION

This study showed that zinc supplementation with 50 mg daily for two months could increase serum level of zinc significantly, but we did not observe any changes in susceptibility of serum lipid to oxidation by either 25 mg or 50 mg zinc supplementation in type 2 diabetic patients.

In diabetes, oxidative stress is due to a higher production of plasma free radical concentrations and a significant reduction in antioxidant defenses (4). Some studies showed that diabetic patients are more prone to oxidative stress because of hyperglycemia (12). It was found that zinc has antioxidant properties and could restrict development and progression of some chronic diseases (13).

Insulin resistance contributes in elevation of lipid peroxidation and free radical production (14). It is hypothesized that zinc could enhance insulin sensitivity in type 2 diabetes (15), which is associated with improved antioxidant status (16). Also some animal studies showed that zinc has a role in insulin synthesis and activity (7,17).

Some studies have shown that zinc supplementation could reduce lipid peroxidation in diabetic patients (10,18,19). In a study by Roussel et al. (19) 56 type 2 diabetic patients with HbAlc more than 7.5% were supplemented with zinc gluconate (containing 30 mg/day zinc) or placebo for six months. The results of their study showed that zinc supplementation caused a decrease in lipid peroxidation monitored by thiobarbituric acid reactants (TBARS) in type 2 diabetic patients. This beneficial effect was also seen in type 1 diabetic patients, who received 30 mg/day zinc as zinc gluconate for three months. There was a reduction in lipid peroxidation and an improvement in antioxidant status (18).

In another study by Fraue et al. (10) 22 type 1 diabetic patients received placebo for 3 months, followed by 30 mg/day zinc gluconate. At the end of the study TBARS decreased significantly after zinc supplementation.

This study, in contrast to some previous studies, could not show any significant improvement in lipid peroxidation with zinc supplementation. It might be due to different duration of zinc supplementation, which was shorter in our study compared with studies mentioned.

Similar results were observed in Blostein-Fujii et al. study which was performed on forty postmenopausal type 2 diabetic women with zinc deficiency. The subjects were allocated to receive placebo or zinc (30 mg/day as amino acid chelate) for three weeks. There was no significant difference in lipoprotein oxidation between two groups (20).

This result was also noted in non-diabetic subjects in a randomized crossover trial. There were no changes in the oxidizability

of LDL or the lipids plasma concentrations following zinc supplementation, 50 mg zinc as 220 mg zinc sulphate daily for 4 weeks, compared to placebo (21).

In conclusion, our findings showed zinc supplementation with 50 mg daily for two months could increase serum level of zinc significantly, but this change was not observed with dose of 25 mg daily in similar duration. This study demonstrated no significance change in serum lipid oxidation parameters in two groups before and after intervention. It seems that parameters like zinc dose, duration of zinc consumption, age, sex, serum zinc status, diabetes status, etc. can be effective in lipid oxidizability.

We propose that these doses are consumed in longer period and also zinc status is evaluated in areas that diabetes is abundant.

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