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Original article

Zinc supplementation attenuate diabetic indices in patients with diabetic retinopathy

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Summary. *Aim:* Zinc (Zn) deficiency has been shown to be associated with age-related eye diseases, such as diabetic retinopathy. Blood sugar and blood pressure are prognostic factors in diabetic retinopathy. The aim of this study was to evaluate the effects of zinc supplementation on diabetes indices and blood pressure in patients with non-proliferative diabetic retinopathy. *Patients and Methods:* 45 patients with non-proliferative diabetic retinopathy (20 males and 25 females) aged from 40 to 70 years were participated in current double-blind randomized placebo-controlled trial. The disease was diagnosed by an expert ophthalmologist according to early photocoagulation and diabetic retinopathy criteria. The patients were assigned to intervention (23 patients) and placebo (n = 22) groups. The groups received zinc (as zinc gluconate; 30 mg) and placebo (maltodextrin; 30 mg) for 3 months, respectively. Fasting insulin, fasting glucose, insulin resistance index (HOMA-IR), hemoglobin A1c (HbA1c), systolic blood pressure (SBP), diastolic blood pressure (DBP) and serum zinc levels were measured before and after the intervention in the both groups. *Results:* Zinc supplementation significantly increased serum zinc ($P_{=}$ 0.004) and decreased serum insulin levels ($P_{=}$ 0.008), fasting blood sugar (FBS) ($P_{=}$ 0.011), HOMA-IR (P= 0.002) and SBP ($P_{=}$0.017) in the intervention group compared with placebo group. The intervention had no significant effects on DBP, HbA1c. *Conclusion:* Due to the positive effects of zinc supplementation on prognostic factors of diabetic retinopathy, it seems complementary therapy with zinc is needed in these patients.

Key words: Diabetic retinopathy, Zinc, Insulin, HbA1c, Blood pressure

Introduction

Diabetic retinopathy is one of the microvascular complications of diabetes. Progression of non-proliferative diabetic retinopathy causes rupture of newly formed blood vessels, vitreous hemorrhage, retinal detachment and ultimately fibrosis (proliferative diabetic retinopathy) (1). The complication involves 80% of more than 10 year diabetics. Despite effective treatments, diabetic retinopathy is still the leading cause of blindness (2).

Many factors have been identified as prognostic factors for this complication which SBP, control blood sugar and nutrient deficiencies are more important prognostic factors of diabetic retinopathy which play a pivotal role in the progression of non-proliferative diabetic retinopathy and subsequently proliferative retinopathy (3-5). It has been suggested that blood sugar and blood pressure serve as prognostic factors in occurrence of diabetic retinopathy (6).

Zinc is one of the micro-nutrients that possess an important role in various metabolic reactions. Impairment in zinc metabolism could be possible causes of blood pressure and blood sugar increment in diabetic patients. On the other hand zinc is accumulated with high concentrations in ocular tissue, particularly

in the retina and choroid. Recent studies have shown that zinc deficiency has role in pathogenesis of cataract, age-related eye damage and even diabetic retinopathy (7). Jyothirmayi et al. in a cross sectional study showed that a serum levels of zinc and sugar are lower and higher respectively in patients with diabetic retinopathy compared with patients without diabetic retinopathy (8). Further, it is known that as diabetic retinopathy progresses serum zinc level reduce and FBS increase (5). There is a vicious cycle between zinc metabolism and diabetes, so that diabetes increases the excretion of zinc and zinc deficiency exacerbates diabetes complications (9, 10). Considering the importance of control of diabetes indices in positive prognosis of diabetic retinopathy and also absence of any study that evaluated the effects of zinc supplementation in diabetic retinopathy, the aim of this study was to evaluate the effects of zinc supplementation on serum insulin, FBS, insulin resistance index, HbA1c and blood pressure in patients with diabetic retinopathy.

Patients and Methods

Study design

This study is a double-blind RCT that was carried out among patients with non-proliferative diabetic retinopathy referred to diabetes clinic of Imam Khomeini and Alavi hospitals, under the supervision of Ardabil University of Medical Sciences, in Ardabil, North West of Iran. The study design and protocol has been approved by the ethic committee of Tabriz University of medical sciences and written informed consent was obtained from patients prior participation in the study. This study was registered in the Iranian Registry of Clinical Trial (www.irct.ir) with registration number ID: IRCT2014040617150N1.

The study's inclusion criteria were as follows: ages between 40 to 70 years and having diabetic non-proliferative retinopathy according to the early photocoagulation for diabetic retinopathy (ETDRS) and diabetic retinopathy study (DRS) criteria. Exclusion criteria were as follows: having cardio vascular disease, kidney or liver disease, taking drugs interfering with the serum level of, taking insulin, being pregnant and lactating, alcoholism, AIDS, infectious diseases and taking zinc containing dietary supplements three months prior participation in the study.

In this study, from total of 180 diabetic patients examined, 50 patients were diagnosed with non-proliferative diabetic retinopathy and enrolled in the study. During the study period, 2 persons in the supplement group and 3 person in control group were excluded due to changes in medications and hospitalization. Thus, the study was carried out with 23 patients in the intervention group and 22 patients in the placebo group. The study subjects were assigned into intervention (23 patients) and placebo (n = 22) groups by block randomization to receive zinc (zinc gluconate; 30 mg) and placebo (maltodextrin; 30 mg) for 3 months, respectively. Two treatment groups were matched by age, gender. Zinc gluconate and placebo tablets were prepared by Jalinous pharmaceutical company (Tehran, Iran) and department of pharmaceutics at Tabriz university of Medical Sciences, respectively.

Dietary and anthropometric assessment

At baseline, end of sixth week and end of the study, 24-hour dietary recall was completed. Further, before and after the intervention anthropometric parameters (height, weight, waist and hip circumference) were measured and BMI (body mass index) and WHR (waist to hip ratio) were calculated. SBP and DBP also were measured before and after the study. WHR is the ratio of the circumference of the waist to that of the hips. BMI was defined as the body weight (kg) divided by the square of the body height (m).

Physical activity

Physical activity was evaluated in MET-minutes/week using international physical activity questionnaire (IPAQ) at first, middle and end of the study.

Biochemical assays

Fasting blood samples were obtained from all of the participants at the beginning and end of the trial. The serum and plasma samples were separated by centrifugation at 2500 rpm for 10 minutes at room temperature. The serum samples were stored at -70°C immediately until their assays. Insulin, HbA1c, FBS and serum zinc were measured using turbidimetry, chemiluminescence, enzymatic colorimetric and

atomic absorption spectrophotometry methods, respectively. Insulin resistance index was calculated using the HOMA-IR formula (fasting plasma glucose (mmol/l) multiplied by fasting serum insulin (mU/l) divided by 405).

Statistical assays

Statistical analysis was performed by SPSS software (SPSS Inc., Chicago, IL, USA). Quantitative and qualitative data were presented as mean ± standard deviation and frequency (percent) respectively. For data analysis, independent sample t-test, Paired t-test, chi-square, linear regression and repeated measure test were used. For confounder adjustment, Analysis of covariance (ANCOVA) was used.

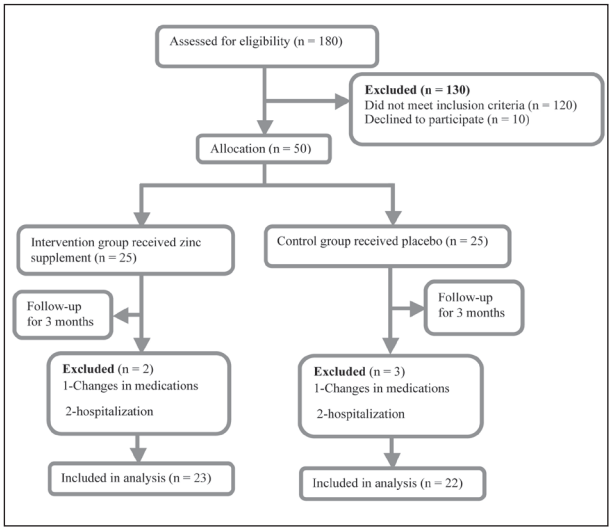

Figure 1. Flow diagram of subject recruitment.

Results

The flowchart of the study has been shown in Figure 1. Totally, 45 patients completed the study. General characteristics of the study participants are presented in Table 1. Age, gender, smoking status, diabetes duration and physical activity levels were not different between the groups. Further, no significant changes were observed in dietary energy, protein, fat, carbohydrate and zinc during the study period (Table 2). The changes of HbA1c, DBP, BMI and WHR were not statistically significant after the zinc treatment as compared with placebo receiving group. Serum concentrations of insulin significantly reduced after treatment with zinc (P<0.05) but not in the placebo group. Levels of FBS in the zinc treated group reduced from 182.04 ± 64.88 to 162.95 ±48.44. Also, SBP decreased from 129.17±14.73 to 125.43±13.57. However, serum zinc increased from 69.91±6.16 to 75.78±7.24. There was a reduction in HOMA-IR in this group (P= 0.002). None of these changes occurred in placebo-treated group. In comparison of mean differences between before and after intervention values of mentioned variables, mean reduction of insulin, FBS, zinc, HOMA-IR and not SBP in the zinc treated group were significantly meaningful

Table 1. Participants demographic characteristics

Variables		Groups		P-value
		Intervention	Placebo	
Sex [n (%)]	male	10(43.5)	10(45.5)	0.894°
	female	13(56.5)	12(54.5)	
Smoking [n (%)]	yes	4(17.4)	4(18.2)	0.624°
	no	19(82.6)	18(81.8)	
Physical activity [n (%)]	low	17(73.9)	19(86.4)	0.459°
	moderate	6(26.1)	3(13.6)	
Age (mean± SD)		57.43±5.75	57.95±5.89	0.766#
Diabetes duration (mean± SD)		8.78±4.67	9.86±4.95	0.456#
BMI (mean± SD)		27.71±4.60	28.79±4.77	0.444#
WHR (mean± SD)		0.96±0.06	0.99±0.04	0.103#

°*P-value based on chi-square test; #P-value based on independent sample T test*

Table 2. Assessment of dietary factors (mean± SD) during the study

Variables		Time points			P-value•
		before	middle	After	
Energy (Kcal)	Intervention	1495.47±581.05	1526.35±427.01	1557.14±631.62	0.256
	Placebo	1447.23±439.31	1385.10±480.19	1598.63±401.36	0.156
	P-value#	0.756	0.302	0.795	
Zinc (mgr)	Intervention	5.52±2.86	5.73±2.81	5.81±4.01	0.286
	Placebo	4.58±3.00	5.82±3.23	5.24±2.88	0.723
	P-value#	0.288	0.916	0.591	
CHO (gr)	Intervention	242.49±124.98	255.34±90.98	252.62±138.36	0.614
	Placebo	244.45±91.55	245.38±77.28	276.13±98.18	0.748
	P-value#	0.952	0.695	0.517	
FAT (gr)	Intervention	31.81±19.20	31.92±14.92	34.32±27.07	0.298
	Placebo	28.52±20.24	34.31±17.19	31.35	0.198
	P-value	0.578	0.621	0.656	
Protein (gr)	Intervention	62.59±27.72	60.62±19.53	62.40±26.62	0.386
	Placebo	55.43±20.28	62.02±24.07	60.35±14.19	0.137
	P-value#	0.330	0.831	0.750	

•P-value based on repeated measure test;# P-value based on independent sample t test

in comparison of placebo-treated group (Table 3). Although the study results revealed zinc supplementation have positive effects on the biochemical parameters but evaluation of the association of before intervention serum zinc with biochemical parameters showed no significant association (Table 4).

Discussion

Blood sugar is one of the prognostic factors in diabetic retinopathy. Control of prognostic factors plays a significant role in progression of non-proliferative diabetic retinopathy to proliferative diabetic retinopathy (3, 5). In this study, zinc supplementation resulted in a significant reduction in insulin, FBS levels and HOMA-IR in patients with non-proliferative diabetic retinopathy. There is any study to evaluate supplementation with zinc in patients with diabetic retinopathy. It has been known that progression of diabetic retinopathy leads to depletion of zinc deposits in the body and exacerbate hyper-glycaemia (5, 11). Ishihara *et al*, in a cross-sectional study, to assessed blood sugar levels in patients with diabetic retinopathy revealed that blood glucose levels in these patients was higher than those without retinopathy (12). In a similar study ganiger *et al* showed that not only blood sugar levels in patients with diabetic retinopathy was higher than those without retinopathy, but the serum zinc of the patients was less than control group. Also the authors reported an inverse association of serum zinc and HbA1c (13).

Many studies reported imbalance between oxidant and antioxidant status in people with diabetes. The improvement in antioxidant status may have role in enhancing insulin action. Accumulation of free radicals may play a pivotal role in complications of diabetes potentially. Among the factors causing free radicals formation can be noted to hyperglycemia, hyperinsulinemia and or insulin resistance (14, 15). Roussel et al. in an interventional study showed that supplementation with zinc improves antioxidant status in patients with type 2 diabetes (16). Zinc plays a key role in the stability of the insulin structure. This micronutrient stores insulin by linking insulin containing vacuoles to cell membranes. On the other hand the element is a powerful antioxidant for oxidizing agents removal (17, 18). Given the role of oxidative stress in initiation and progression of diabetes and insulin resistance, zinc deficiency can have a central role in diabetes complications (19, 20).

Table 3. Diabetic indices (Mean ± SD) following zinc treatment

Variables		Groups				P-value•
		Intervention		Placebo		
		Mean ± SD	Percent change	Mean ± SD	Percent change	
Insulin (mIU/ml)	Before	8.47±4.84	-16.84	10.62±3.25	-5.14	0.008
	After	6.42±3.64		10.07±3.85		
	P-value#	0.007		0.324		
FBS (mg/dl)	Before	182.04±64.88	-7.65	194.31±45.34	4.64	0.011
	After	162.95±48.44		200.27±55.9		
	P-value#	0.012		0.549		
HOMA-IR	Before	3.80±2.44	21.04	5.07±1.81	-0.32	0.002
	After	2.50±.38		5.14±2.89		
	P-value#	0.002		0.879		
HbA1c (%)	Before	8.54±1.78	-2.48	8.90±1.54	3.36	0.079
	After	8.22±1.57		9.02±1.27		
	P-value#	0.250		0.671		
SBP (mm Hg)	Before	129.17±14.73	-2.61	126.72±12.25	2.40	0.017
	After	125.43±13.57		129.40±11.44		
	P-value#	0.048		0.167		
DBP (mm Hg)	Before	74.73±9.97	-2.12	76.50±8.70	2.85	0.195
	After	72.65±9.85		78±7.95		
	P-value#	0.236		0.490		
BMI (kg/m^2)	Before	27.71±4.60	-0.07	28.79±4.77	0.30	0.396
	After	27.67±4.53		28.92±5.11		
	P-value#	0.813		0.258		
WHR	Before	0.96±0.06	0.07	0.99±0.04	-0.30	0.396
	After	0.96±0.05		0.95±0.19		
	P-value	0.619		0.353		
Zinc (µgr/dl)	Before	69.91±6.16	8.69	75.42±7.83	-1.49	0.004
	After	75.78±7.24		74.14±8.43		
	P-value#	<0.001		0.329		

•P-value based on ANCOVA after adjustment for baseline values, age and sex; #P-value based on Paired Sample T test

Table 4. The relation between zinc and biochemical parameters and blood pressure before intervention

Variables	B	P-value*	95% confidence interval	
			Lower	Upper
Insulin	-0.019	0.403	-1.336	3.254
FBS	0.959	0.738	-0.131	3.254
HOMA-IR	-2.649	0.312	-7.886	2.588
HbA1c	1.739	0.364	-2.091	5.569
SBP	-0.179	0.202	-0.457	0.100
DBP	0.261	0.184	-0.130	0.653

**Reported based on linear regression*

Blood pressure, like blood sugar, is one of the prognostic factors in patients with diabetic retinopathy. Control of blood pressure in people with diabetes can prevent the exacerbation of microvascular complications (21). In this study, zinc supplementation significantly reduced SBP. There is no report to evaluate the effects of supplementation with zinc in patients with diabetic retinopathy. Schrier et al. in a five years follow up prospective study demonstrated control of blood pressure significantly reduce diabetic retinopathy progression (22). In another cohort study Muir et al. reported that

hypertension augments progression of diabetic retinopathy (OR: 2.16; 95%CI: 1.03-4.52) (23). Also such association was reported by porta et al (OR: 1.50; 95%CI: 1.03-2.20) (21). As diabetes progresses zinc levels in serum tends to decrease. Many studies have shown the association of zinc with high blood pressure (5). Chiplonkar et al. revealed that serum levels of zinc in subjects with hypertension was significantly lower than normotensive ones (24). Jayawardena et al. in a meta-analysis assessed the effects of zinc supplementation on diabetic indices and concluded that supplementation with zinc reduce SBP not DBP (25). Renin-angiotensin system activity regulation could be one of the reasons of inverse association between zinc and blood pressure. One of the main causes of hypertension is activation of renin-angiotensin system (17, 26). Tubek et al. in a cross sectional study revealed a negative correlation between zinc content of blood leukocyte and the activity of renin-angiotensin system (27). Renin cleaves a peptide, called angiotensinogen, into angiotensin I by angiotensin-converting enzyme. Angiotensin I converts to angiotensin II and the latter binds to receptors on specific cells that surround blood vessels and stimulates their contraction to increase blood pressure (28, 29). Zinc also plays a role in maintaining the structure and function of more than 300 types of proteins. Angiotensin-converting enzyme is one of the proteins that zinc control its function using negative feedback (18, 30).

Conclusion

Zinc supplementation in this study significantly improved insulin resistance and blood pressure in diabetic patients with non-proliferative retinopathy. According to the results, supplementation with zinc is effective to control the progression of diabetic retinopathy.

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