

## **Relationships between serum zinc concentrations and inflammatory intermediate factors, blood glucose, and HbA1c in postmenopausal diabetic women**

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### **Abstract**

**Background:** Zinc (Zn) is one of the essential nutrients that its metabolism changes in diabetes. It has been shown that there is a relationship between Zn and both kinds of diabetes [non-insulin-dependent diabetes mellitus (NIDDM) and insulin-dependent diabetes mellitus (IDDM)]. TNF- $\alpha$  and IL-6 relate with BMI and insulin resistance. It seems that there are relationships between Zn and TNF- $\alpha$ , IL-6, blood glucose and HbA1c. The aim of present study was to investigate relationship between serum zinc concentration and inflammatory intermediate, blood glucose and HbA1c in postmenopausal diabetic women.

**Methods:** We studied 45 diabetic women and 45 healthy women (controls) with BMI 25-30 kg/m<sup>2</sup> and aged 45-60 years. Fasting blood sugar (FBS), HbA1c, serum zinc, TNF- $\alpha$ , and IL-6, were determined in the two groups.

**Results:** There were non significant positive correlations between Zn, IL-6 ( $r=0.28$ ) and TNF- $\alpha$  ( $r= 0.03$ ) and a non significant negative correlation between Zn and FBS ( $r= -0.06$ ) and HbA1c ( $r= -0.07$ ) in diabetic group.

**Conclusion:** There were not a significant relationship between serum Zn and inflammatory intermediates (TNF- $\alpha$ , IL-6), FBS, and HbA1c in postmenopausal diabetic women.

**Keywords:** Type 2 diabetes mellitus, Zinc, Inflammatory intermediate factors, FBS

## Introduction

Type 2 diabetes includes about 90 to 95 percent of all diagnosed diabetes cases. The most important risk factors for type 2 diabetes are high energy intake, aging, inactivity (sedentary life-style) and obesity (1). Among diabetes complications are blindness, kidney disorders, hypertension, cardiovascular diseases and foot amputations (2, 3). Glycosylated hemoglobin (Hb A1C) is used for surveillance of long-term blood glucose control (in the past 2-3 months) (4).

Zinc (Zn) is one of the essential nutrients which its metabolism changes in diabetic patients (5). There is a relationship between Zn and insulin dependent diabetes mellitus (type 1 diabetes) and non-insulin dependent diabetes mellitus (type 2 diabetes) (6; 7). Zn is needed for growth and development, for the proper functioning of the immune system, for carbohydrates, lipids, proteins and nucleic acids metabolism, reproduction, bone mineralization and gene expression (8-11).

Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) is a cytokine that is largely secreted from monocytes and macrophages. This cytokine causes many cellular and metabolic changes in critically ill patients (9). TNF- $\alpha$  increases in obesity-induced insulin resistance, which shows that TNF- $\alpha$  may play a role in insulin resistance (12).

Also, Interleukin-6 (IL-6) is a cytokine that mainly produced by fat tissue. Circulating IL-6 levels correlate with body mass index (BMI), insulin sensitivity and glucose tolerance (9). Some studies have shown that Zn levels are affected by to inflammatory Factors; TNF- $\alpha$  and IL-6 (13, 14). TNF- $\alpha$  and IL-6 influence the metallothionein homeostasis, which has an important role in the Zn homeostasis (15, 16). It seems that further studies in this field, especially in diabetic patients are warranted.

Because of increase in prevalence of diabetes mellitus and the importance of Zn in diabetic patients through its relationship with inflammatory factors and blood sugar, it is important to study the relationship between Zn and these factors in these patients. This study investigated the relationship between serum Zn concentration and inflammatory factors (TNF- $\alpha$  and IL-6) and blood sugar (glucose and HbA1c) in postmenopausal diabetic and healthy women.

## Methods

This was a cross-sectional study. The participants were 45 women with type 2 diabetes mellitus, who had been referred to the Iranian Diabetes Society, and 45 women without diabetes. The ranges of age and body mass index (BMI) were similar in diabetic and healthy groups. The method of sampling was convenient sampling. The inclusions criteria were: type 2 diabetes [fasting blood sugar (FBS) greater than 126 mg/ dl] at least for 3 years after diagnosis (not for healthy group), aged 45-60 years and BMI 25-30 kg/m<sup>2</sup>. All the patients were taking oral hypoglycemic drugs only. None of the subjects suffered from chronic diseases (cardiovascular, kidney and thyroid disorders). They were not receiving blood cholesterol lowering drugs and Zn supplements. Written informed consent was obtained from all subjects. After fasting for 12 hrs and before receiving oral hypoglycemic drugs, 10 ml blood samples were collected. In order to obtain plasma and to avoid clotting, 2 cc of blood samples were put in a tube containing EDTA 5% (0.3 ml). The blood was centrifuged at 1500 g for 10 minutes, and plasma was separated, then red blood cells were washed with normal saline three times to take hemolization to measure HbA1c. Colorimetric assay was used (M. Parker method) to determine HbA1c (17). We put the rest blood samples (8 cc) in another tube without anticoagulant. Firstly, the tube was placed in laboratory temperature for 0.5 h and then was centrifuged at 1500 g for 10 minutes to separate serum. Serum glucose was measured by using enzymatic method (Zistshimi kit, Tehran, Iran). Serum Zn was determined using enzyme-linked immunosorbent assay (ELISA) (Randox kit, London, UK). TNF- $\alpha$  was measured using ELISA (Bender MedSystem kit, Vienna, Austria). The intra-assay analytical coefficient of variation was 6% and sensitivity was 2.3 pg/ml. IL-6 was measured using ELISA (Bender MedSystem kit, Vienna, Austria). The intra-assay analytical coefficient of variation was 3.4% and sensitivity was 0.92 pg/ml.

## Statistical analysis

All values are represented as Mean $\pm$ standard error (Mean  $\pm$  SE). Independent T-test was used to compare continuous variables between two groups. Pearson Correlation Coefficient was

used to evaluate the relationship between quantitative variables. The p-value less than 0.05 was considered to be statistically significant.

## Results

There were not any statistically significant differences between the mean of age, BMI and duration of menopause in diabetic and healthy women (Table 1). The mean value of glucose and HbA1c were significantly less in healthy women compared to diabetic women ( $p < 0.001$ ) (Table 1). The mean of serum Zn concentration was higher in healthy women but it was not significant. IL-6 and TNF- $\alpha$  were higher in

diabetic women compared to healthy women ( $p > 0.05$  and  $p < 0.001$ , respectively) (Table 1). There was a negative relationship between serum Zn and serum glucose concentrations on one hand and serum Zn concentration and HbA1c on the other in both groups, but the result was not significant (Table 2). Serum Zn concentration was positively correlated with IL-6 in diabetic women and was negatively correlated with IL-6 in healthy women, but it wasn't significant in either group (Table 2). Also, there was a positive relationship between serum Zn concentration and TNF- $\alpha$  in both diabetic and healthy women, but it was only significant in healthy women ( $p = 0.03$ ) (Table 2).

**Table 1. General characteristics and serum parameters of diabetic and healthy women**

Variables	Diabetic subjects (n=45)	Healthy subjects (n=45)
Age (years) **	54.13 $\pm$ 0.44 <sup>†</sup>	52.78 $\pm$ 0.72
BMI (kg/m <sup>2</sup> ) **	27.67 $\pm$ 0.26	27.47 $\pm$ 0.3
Duration of menopause(y) **	4.96 $\pm$ 0.36	5.56 $\pm$ 0.57
Glucose (mg/dl) *	168.62 $\pm$ 7.5	93.76 $\pm$ 1.7
HbA1c (%)*	8.61 $\pm$ 0.24	6.23 $\pm$ 0.12
Zinc ( $\mu$ g/dl)**	94.07 $\pm$ 3.4	99.24 $\pm$ 2.5
IL-6 (pg/ml)**	2.3 $\pm$ 0.25	1.7 $\pm$ 0.21
TNF- $\alpha$ (pg/ml)*	4.3 $\pm$ 0.22	2.9 $\pm$ 0.15

A cross-sectional study, Statistical analysis: t-test,<sup>†</sup> Values are means $\pm$  SE.

BMI=body mass index;

\* P < 0.01 statistically significant.

\*\* Non significant

**Table 2. Correlations between serum Zn levels and other parameters**

Variables	Diabetic subjects (n=45)	Healthy subjects (n=45)
	r	r
Glucose	-0.06**	-0.08**
HbA1c	-0.07**	-0.008**
IL-6	0.28**	-0.24**
TNF- $\alpha$	0.036**	0.31*

A cross-sectional study, Statistical analysis: Pearson correlation coefficient

\* P < 0.01 statistically significant.

\*\* Non significant

## Discussion

This study investigated the possible relationships between serum Zn concentration and inflammatory factor, TNF- $\alpha$  and IL-6 and blood sugar (glucose and HbA1c) in postmenopausal diabetic and healthy women. In this study there was not any negative relationship (correlation) between serum Zn concentrations and serum glucose in diabetic women. Several studies have shown that there is a negative relationship between serum Zn concentration and serum glucose levels. In these studies Zn deficiency elevated glucose concentration and Zn supplement decreased it

(18, 19). Zn plays an important role in insulin synthesis and function, insulin connection to the cells (20), and glucose entrance to the cells (21). Also, Zn is a main cofactor in some glucose metabolism enzymes and as a result, its deficiency can disrupt carbohydrates metabolism (22, 23). Zn deficiency can complicate glucose transportation into the cells, by disrupting lipid metabolism and membrane flexibility (22). Some studies have demonstrated that Zn supplement reduces lipid peroxidation and oxidative stress in diabetic patients, although it does not significantly change glucose levels (7, 24). Zn is powerful

antioxidants and its deficiency increases oxidative damages to various organs (25, 26); nevertheless, hyperglycemia may elevate lipid peroxidation and oxidative stress in diabetic patients (27) that could lead to cardiovascular complications in these patients (28). Therefore, Zn has an possible role in improving diabetic patients through reducing glucose levels and oxidative stress.

Our result showed that there is a non significant negative relationship between serum Zn levels and HbA1c in diabetic women. Several studies have demonstrated that there is a negative relationship between Zn and HbA1c in diabetic patients, and Zn supplements decrease HbA1c in those patients (7, 18, 29). In one study, Zn supplement had no significant effect on HbA1c in diabetic patients, but reduced oxidative stress. Perhaps Zn effects on oxidative stress are more appreciable and more sensitive than its effects on other variables and taking Zn supplement for a longer time period can decrease HbA1c (24).

In our study, there was a non significant positive relationship between serum Zn levels and IL-6 concentration in diabetic women. Some studies have shown that Zn negatively correlated with IL-6 (13). IL-6 accelerates metallothionein gene expression and its production (30). IL-6 affects Zn transportation and metallothionein homeostasis in inflammation and aging (16). Metallothionein has an important role in regulating Zn homeostasis (31). The important function of metallothionein is releasing Zn in response to the oxidative stress when Zn-dependent antioxidant enzymes are necessary (32-34). This response is reduced in chronic inflammation and aging (35). Increased IL-6 levels is related to hyperglycemia, elevated LDL and triglyceride levels and insulin resistance in patients with type 2 diabetes (36). There is chronic inflammation in type 2 diabetes patients due to high IL-6 production and Zn deficiency correlates with inflammation, atherosclerosis and type 2 diabetes (16, 37, 38). Consequently, higher bioavailability of Zn ion, with its antioxidant property, can prevent increasing free radicals and inflammation (39). Increased IL-6 production can influence

metallothionein homeostasis and compromises Zn bioavailability, at the same time, metallothionein homeostasis and regulation of circulating Zn levels may affect IL-6 production (40). The relationship between IL-6 and Zn seems to be mutual: increased IL-6 gene expression changes Zn homeostasis and reduces Zn bioavailability (40) and consuming Zn containing foods can reduce IL-6 and thus improve the inflammation (41). In this study, we found a positive relationship between Zn levels and IL-6 in diabetic women, maybe, because of normal Zn levels in these patients.

According to our findings, there was a non significant positive relationship between serum Zn and TNF- $\alpha$  levels in diabetic women. Studies have been reported that Zn positively correlated with TNF- $\alpha$  (14). TNF- $\alpha$  affects metallothionein gene expression, like IL-6(15) and, metallothionein plays an important role in increasing the immunity by improving Zn bioavailability (31). Mariani et al. found that there is a negative relationship between Zn and TNF- $\alpha$  (42), they concluded that Zn deficiency and higher ages of the samples could be the underlying reason for getting such results. Zinc plays an important role in different genes expression, but its role in regulating TNF- $\alpha$  gene expression is not clear until now and further studies are needed.

The present study was a cross sectional one. Therefore, we can not conclude any causal relationship between Zn and other variables. In conclusion, there were not significant relationships between serum Zn concentration and inflammatory factors, IL-6 and TNF- $\alpha$  and blood glucose and HbA1c in postmenopausal women with type 2 diabetes. It seems that these relationships need to be further clarified by future studies with larger sample sizes and more advanced designs.

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