Assessment the effect of Zn and Mg supplementation on total antioxidant capacity in type 2 diabetic patients

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Abstract

In the presesnt study, the effect of Zn & Mg oral supplementation on total antioxidant capacity (TAC) in diabetic patients was determined using two control groups (group A represent healthy control and group B represent diabetic control), and two treatment groups of diabetic patients. C-group was supplemented 50mg/day of Zn as sulfate, while D-group treated with 300mg/day of Mg as citrate within 2 months period. The results show that there was a significant decreases ($P < 0.01$) in serum TAC level in type 2 DM subjects (Group B) & (C and D) compared with that of healthy control (Group A). Also, there was a significant increases ($P < 0.01$) in TAC level in group C and D, compared with group B. While, no significant changes has been shown in serum TAC level between group C and D of diabetic subjects.

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially eyes, kidneys, nerves, hearts and blood vessels (Peerapatdit *et al.,* 2006). Age-related degenerative changes affect a wide range of biological tissues. Accelerated aging changes that contribute to early mobidity are common in type 2 diabetes mellitus (DM) (Pietropaolo and Roith, 2001).

Oxygen homeostasis (pro-oxidant and antioxidant) is of critical importance for maintaining tissue viability and function. Oxidative stress may occur due to an increase in free radical production and/or a decrease in antioxidant defenses. Autoxidation of glucose and glycated proteins, activation of polyol pathway, increased intracellular nicotinamide

adenine dinucleotide hydrogen/nicotinamide adenine dinucleotide ($NADH/NAD^+$) ratio, altered cell glutathione and ascorbate redox status as well as perturbations in nitric oxide and prostaglandin metabolism are the main mechanisms underlying oxidative stress in diabetes (Gul *et al.,* 2003). Blood contains many antioxidant molecules which prevent and/or inhibit harmful free radical reactions. Exogenous antioxidants, like vitamin C and vitamin E. Endogenous antioxidant like albumin, bilirubin, uric acid, superoxide dismutase and glutathione peroxidase as the scavenger enzymes can protect the cell against the potentially harmful effects of oxidant agents (Young and Woodside, 2001). Concentration of these antioxidants in the serum can be measured one by one, but this procedure is time-consuming, labour-intensive, costly and requires complicated techniques (Erel, 2004**a**). On the other hand, total antioxidant capacity (TAC) whose measurement method has been recently specified and developed can reflect the total antioxidant state of the serum (Ghiselli *et al.,* 2000). The aim of this study is to evaluate the effect of zinc and magnesium supplementation on total antioxidant capacity (TAC) in diabetic patients and comparison with diabetic patients without intake of these supplements.

Materials and Methods

Chemicals

The following chemicals have been supplied by Sigma - Aldrich Co. (St. Louis, MO)**:** Ethylenediamin tetra-acetic acid (EDTA), Ammonium ferrous sulfate, $ZnSO_4$, KH_2PO_4, Na_2HPO_4 and Uric acid**.** E. Merck (Darmstadt, Germany) supplied the following chemicals**:** Glacial acetic acid, H_2O_2, Mg-citrate, Na-Benzoate, NaOH, and Thiobarbituric acid**.** All of the reagents were prepared in deionized distilled water (ddH_2O) to eliminate the contamination of metal ions.

Samples preparation

This study was involved 60 patients suffering from type 2 diabetes (25 male and 25 females), age (35-65) years old (mean ± SD = 51.96 ± 10.13), while the controls population involved 30 healthy indivduals (15 male and 15 females), whose age was (25-65) years old (mean ± SD = 42.66 ± 11.46). The study samples were divided into four subgroups as follow:

- Group A : contain 30 healthy people (15 male and 15 females) aged between (25-65) years.
- Group B : contain 20 patients with type 2 (10 male and 10 females) aged between (35-65) years.
- Group C : contain 20 patients with type 2 (9 male and 11 females) aged between (35-65) years. This group taken Zn supplementation as zinc sulfate, (50 mg/day) for two months continuously.

◘ Group D : contain 20 patients with type 2 (8 male and 12 females) aged between (35-65) years. This group taken Mg supplementation as magnesium citrate, (300 mg/day) also two months continuously.

After 8-12 hours of fasting, blood was drawn by veinpuncture at the antecubital vein from the healthy and subjects recently diagnosed with type 2 diabetes in Basrah Governorate, AL-Seadey Special Hospital. After clotting, serum was separated by centrifugation (402 Xg for 10 min.) and divided in several aliquots, stored in deep freeze at (-20 °C) until the time of measurement.

Measurement of Total antioxidant capacity

Total antioxidant capacity (TAC) was measured according to the method of (Koracevic *et al.,* 2001). This method is used to measure the reductive power of a sample. It is based on suppression of the production of TBARS (Thiobarbituric acid reactive substances) in the presence of antioxidants. Briefly, the reactive mixture contained 0.5mL of a (10 mmol/L) Na-Benzoate, 0.2mL of H_2O_2 (10 mmol/L), 0.49 mL of phosphate buffer (100 mmol/L, pH=7.4) (prepared by mixing 19.5 mL of KH_2PO_4 (100 mmol/L) with 80.5 mL of Na_2HPO_4 (100 mmol/L), then adjusted the pH to 7.4) and 0.2 mL of Fe-EDTA complex (2mmol/L) (prepared freshly by mixing equal volumes of EDTA (2mmol/L) and ammonium ferrous sulfate (2mmol/L), then left to stand at 25°C for 60 min). Ten microliters of the blood serum was added to the latter reactive mixture and was incubated at 37°C for 60 min. Finally, 1 mL glacial acetic acid (20 mmol/L) and 1 mL thiobarbituric acid (0.8% w/v in 100 mL of 50mmole/L NaOH) was added snd the absorbance at 532 nm was measured spectrophotometrically after incubation at 100°C for 10 min. Total antioxidant capacity was calculated according to the following formula:

$$TAA\ (mmol/L) = (C_{UA})\ (K - A) / (K - UA)$$

Where:

(C_{UA}) : Concentration of uric acid (mmol/L) .

K : Absorbance of control (K_1 - K_0) .

A : Absorbance of sample (A_1 - A_0) .

UA : Absorbance of uric acid solution (UA_1 - UA_0) .

Statistical analysis

The results are expressed as mean ± standard deviation SD. Comparison between control and diabetic patients were performed by Anova-one way taking ($P < 0.05$) as lowest limit of significance for different quantitative parameters.

Result and Discussion

It was shown that reactive oxygen species (ROS) and other oxidants could be

also formed in the normal physiological process (Onder and Gurer, 2001). In people with diabetes the vulnerability to oxidative damage may be partly attributed to lower antioxidant micronutrient status including trace elements (Chausmer, 1998). In this work, a significant decreases ($P < 0.01$) was observed in serum TAC level in type 2 DM subjects (Group B) & (C and D) compared with that of healthy control (Group A), figure 1, and table 1. Same figure and table reflects the levels of serum TAC in type 2 DM subjects (Group C and D) with their significant increases ($P < 0.01$) compared with type 2 DM control (Group B). While, no significant changes has been shown in serum TAC level between group C and D of diabetic subjects.

Table 1: Level of TAC in serum of healthy control (group A) and diabetic patients (group B, C and D).

			Group A	Group B	Group C	Group D
Serum TAC level(mmol/L)	Mean ± SD		1.74 ± 0.04	0.83 ± 0.03	1.28 ± 0.05	1.10 ± 0.06
	SE		0.008	0.029	0.014	0.012
	Range		0.9 - 2.43	0.4 - 1.6	0.7 - 2.1	0.6 - 1.97
	95% CI	Lower	1.62896	0.46912	1.08568	0.93344
		Upper	1.85104	1.19088	1.47432	1.26656

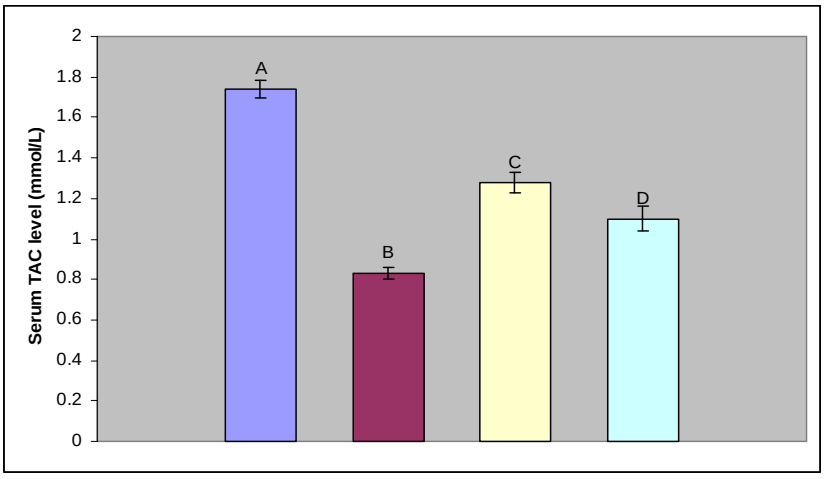

Figure 1: Level of TAC in serum of healthy control (group A) and diabetic patients (group B, C and D).

Many antioxidant molecules found in blood prevent or inhibit the harmful effects of free radicals. Whenever there is a decrease in antioxidants and/or an increase in oxidants,

oxidant/antioxidant balance is impaired in favor of oxidants and this is known as oxidative stress (Isik and Koca, 2006). It is known that oxidative stress is responsible for tissue injury in many diseases and contributes to the development of atherosclerosis (Abuja and Albertini, 2001). Antioxidant activity indicates the antioxidant characteristic of only one antioxidant, whereas total antioxidant capacity (TAC) represent the total antioxidant characteristic of all antioxidants found in plasma. It is doubtlessly more advantageous to evaluate TAC, instead of individual antioxidant activities. Many methods have been developed recently for this aim. Total radical trapping antioxidant parameter (TRAP), oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) are colorimetric methods previously developed to assess TAC (Erel, 2004**a**) (Erel, 2004**b**) (Ghiselli *et al.,* 2000). The potential antioxidant effects of Zn in diabetes could be related to several mechanisms. It has been suggested that Zn metallothionein complexes in islet cells provide protection against immune-mediated free-radical attack. Zinc could act also in protecting sulfahydryl groups against oxidation and participatecycle by competing with transition metals (Anderson *et al.,* 2001) (Bray and Bettger, 1990). The mechanism by which Mg acts as antioxidant is still not totally understood. Several mechanisms including insulin secretion, binding and action have been proposed to explain the effect of intracellular or plasma magnesium on diabetes pathogenesis (Tosiello, 1996). Intracellular magnesium is a critical Co-factor for several enzymes in carbohydrate metabolism, especially those involved in phosphorylation reactions such as tyrosine kinase. Magnesium play important role in the formation of metal-phosphate complexes. The complexes formed play a critical role by activating the biocatalysts involved in the manner in which glucos and triglyceride (Gurreiro and Rodriguez, 2006) (Lopez *et al.,* 2004).

In conclusion, TAC is an appropriate measurement method demonstrating oxidant/antioxidant balance. Oxidant/antioxidant balance seems to be impaired at all stages of diabetes mellitus. In future, we think that this argument should be confirmed by controlled, multi-centered, prospective studies, which shall include large case series and employ reviewed disease activity criteria.

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دراسة تأثير عنصري الزنك والمغنيسيوم كمكملات غذائية على المستوى الكلي لمضادات الأكسدة في مرضى السكري من النوع غير المعتمد على الأنسولين

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الخلاصة

تضمنت الدراسة الحالية دراسة تأثير عنصري الزنك والمغنيسيوم كمكملات غذائية على المستوى الكلي لمضادات الاكسده في مرضى السكري من النوع غير المعتمد على الأنسولين.إذ تم استخدام مجموعتي سيطرة ألمجموعه A(مجموعة الأصحاء) والمجموعة B (مجموعة مرضى السكري)، واستخدام مجموعتي معامله لمرضى مصابين بداء السكر: ألمجموعه-C أعطيت جرعه قدرها (50ملغ/يوم) من الزنك بشكل كبريتات الزنك ، بينما عوملت ألمجموعه-D مع (300 ملغ/يوم) من المغنيسيوم بشكل سترات المغنيسيوم، خلال فتره قدرها شهرين. أوضحت النتائج أن هناك انخفاض معنوي ($P < 0.01$) في المستوى الكلي لمضادات الاكسده عند مرضى السكري (المجموعة B) والمجموعتين المعاملتين مع Zn و Mg (D & C) مقارنة مع ألمجموعه A. كما لوحظ ازدياد معنوي ($P < 0.01$) في المستوى الكلي لمضادات الاكسده في المجموعتين D &C بالمقارنة مع ألمجموعه B، بينما لم يظهر تغير معنوي في المستوى الكلي لمضادات الاكسده بين المجموعتين D &C المعاملتين بالمكملات الغذائية .