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For further information about this article or if you need reprints, please contact:

Abdoljalal Marjani
Golestan University of
Meidcal Sciences, Gorgan, Iran

Tel: 0098-171-4421651,
4421653, 4422652
Fax: 0098-171-4421289

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Plasma Lipid Peroxidation Zinc and Erythrocyte Cu-Zn Superoxide Dismutase Enzyme Activity in Patients with Type 2 Diabetes Mellitus in Gorgan City (South East of the Caspian Sea)

[1]Abdoljalal Marjani, [2]Abdolvahab Moradi and [2]Mohsen Saeedi

The aim of this study was to determine the changes of plasma lipid peroxidation, zinc and erythrocyte Cu-Zn superoxide dismutase activity in patients with type 2 diabetes mellitus and healthy control in Gorgan city. Fifty type 2 patients with diabetes mellitus and 50 people without diabetes were included in this randomized study. None of patients studied had any diabetic complications. The levels of plasma malondialdehyde and zinc from type 2 diabetes mellitus patients (6.24±0.85 nmol mL^{-1} and 116.78±5.51 μg dL^{-1}) and control groups (3.63±0.97 nmol mL^{-1} and 146.86±9.06 μg dL^{-1}) were determined. Erythrocyte Cu-Zn superoxide dismutase activity from patients with diabetes (675.34±60.89 U g^{-1} Hb) and control groups (1052.70± 52.76 U g^{-1} Hb) were determined. The increased plasma lipid peroxidation and decreased plasma zinc and erythrocyte Cu-Zn superoxide dismutase activity that we observed in patients with type 2 diabetes mellitus may predispose to the development of cardiovascular complications. We propose that diabetic patients may have elevated requirement for antioxidants. Supplementation with zinc and vitamin or dietary free radical scavengers such as vitamins E and C or tomato, orange and etc. have a potential role in boosting antioxidant-related defences and maybe important in patients with diabetes.

Key words: Lipid peroxidation-superoxide dismutase-zinc-type 2 diabetes mellitus

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[1]Department of Biochemistry and Biophysics,
[2]Department of Microbiology and Immunology,
Golestan University of Medical Sciences, Gorgan, Iran

INTRODUCTION

Free radicals are highly reactive molecules generated by biochemical redox reactions that occur as a part of normal cell metabolism (Kohen *et al*., 1996). These unstable species may cause oxidative damage to DNA, carbohydrate, proteins and lipids that are normally counteracted by protective antioxidants. Oxidative defense is provided by a number of enzymes and vitamins, including the vitamin E, vitamin C and glutathione (Halliwell, 1997; Halliwell and Gutteridge, 1989; Sies, 1993). In times of increased free radical production, individuals may become deficient in these antioxidants. Excessive free radical production or low antioxidant level leads to oxidation of cellular lipids, proteins and nucleic acids, which results in fragmentation and cross-linking. This may ultimately lead to cell death with widespread pathological consequences (Baynes, 1991) The imbalance between protective antioxidants (antioxidant defense) and increased free radical production, leading to oxidative damage, is known as oxidative stress. Oxidative stress is caused by a relative overload of oxidants, i.e., reactive oxygen species. This impairs cellular functions and contributes to the pathophysiology of many diseases. Evidence has accumulated suggesting that complications of diabetes seem to be partially mediated by oxidative stress (Hayoz *et al*., 1998; Rosen *et al*., 1998; Szaleczky *et al*., 1999). The function of zinc in the body metabolism is based on its enzymatic affinity, such as a zinc-enzyme complex or Zinc metalloenzyme. In humans and animals, diabetes maybe results in disturbance of these vital trace elements (Kinlaw *et al*., 1983). In most mammals, insulin is stored as zinc crystals and is likely secreted in zinc form. Zinc has important role in modulating the immune system and its dysfunction in diabetes mellitus may be related in part to the status of zinc (Mocchegianai *et al*., 1989). Lack or inadequate supply of such nutrients produces functional impairment and can result in disease. The clinical significance and evaluation of zinc in regard to different diseases, including diabetes mellitus remains conflicting as well as controversial. Many questions still remain unanswered. Several reports underscore the role of micronutrient status in patients with type 1 or 2 diabetes mellitus (Mooradian *et al*., 1994; Strain, 1991; Anderson, 1995; Chaumer, 1998; Anderson *et al*., 1997; Ravina *et al*., 1999a, b). Zinc maybe act in normalizing glycemia and a restored zinc status in patients with type 2 diabetes mellitus may counteract the deleterious effects of oxidative stress, helping to prevent many complications associated with diabetes.

Diabetes mellitus is a major source of morbidity in developed countries. Morbidity and mortality are increased in patient with type 2 diabetes mellitus compared with the general population, particularly with respect to coronary artery disease. One possible explanation for this is that oxidative stress may be a contributory factor. There are contradictory reports on changes in plasma lipid peroxidation, zinc and erythrocyte Cu-Zn superoxide dismutase activity in patients with type 2 diabetes. Some of the studies show an increase whereas others show a decrease, or no significant differences. There are a few reports describing differences in plasma lipid peroxidation, zinc and erythrocyte antioxidant enzymes between patients with type 2 diabetes and controls. Zinc as a cofactor of enzyme Cu-Zn superoxide dismutase is an important element for activity of this enzyme. Because of the contradictory results in this field of study, the present study was designed to determine the changes of plasma lipid peroxidation(by measuring the level of MDA), zinc and erythrocyte Cu-Zn superoxide dismutase activity in patients with type 2 diabetes mellitus (at a diabetes center in Gorgan city) in comparison to controls.

MATERIALS AND METHODS

Samples were obtained, in a randomized fashion, from 50 patients with type 2 diabetes as well as 50 control subjects. Patients were chosen from the patients referred to the Department of Diabetes Center at the 5th Azar Hospital in Gorgan University of Medical Sciences. The study was carried out during 2005. The patients studied had no evidence of vascular complications, including hypertension, coronary artery disease. Controls were defined as not having a major medical illness, no hospital admissions, no current medication and a subjective perception of good health as determined by health questionnare. None of the study subjects received any medication and trace element supplement in the previous 2-3 months. Characteristics of both groups are shown in the Table 1. Blood samples were obtained after an overnight fast in heparinized tubes. Plasma was separated soon after blood was taken. The plasma malondialdehyde (the level of lipid peroxidation expressed as malondialdehyde [MDA]), fasting blood sugar (FBS) and erythrocyte Cu-Zn superoxide dismutase (SOD), glycated haemoglobin (HbA_{1c}) and hemoglobin (Hb) were determined using laboratory kits and spectrophotometry techniques (model JENWAY 6105 UV/VIS). Plasma Zinc (Zn) was measured using randox laboratory kit (Homster and Zak, 1985) and spectrophotometry techniques.

Table 1: Baseline characteristic

Characteristic	Patients with diabetes	Controls
No. of subjects	50	50
Age (years)	48.47±6.87	47.66±5.68
Sex		
male	20	22
female	30	28
Duration of diabetes (years)	2.78±0.74	-----
FBS (mg dL^{-1})	204.54±32.42	85.62±8.31
HbA_{1c} (%)	10.67±1.06	6.31±0.83

Plasma MDA, FBS and erythrocyte Cu-Zn SOD, HbA_{1c} and Hb were determined using previously described methods (Satoh, 1978; Barnrnham, 1972a, b; Wooliams *et al*., 1983; Boer *et al*., 1992). Data was analyzed by Student's t-test, using SPSS-10 software. $p<0.05$ was considered significant.

Malondialdehyde measurement: To 0.5 mL plasma, 2.5 mL of trichloroacetic acid was added and the tube was left to stand for 10 min at room temperature. After centrifugation at 3500 RPM for 10 min, the supernatant was decanted and the precipitate was washed once with sulfuric acid. The after, 2.5 mL sulfuric acid and 3 mL thiobarbituric acid (TBA) in sodium sulfate were added to the precipitate and the coupling of lipid peroxide with TBA was carried out in a boiling water bath for 30 min. After cooling in cold water, the resulting chromogen was extracted with 4 mL of n-butyl alcohol by vigorous shaking. Separation of the organic phase was facilitated by centrifugation at 3000 RPM for 10 min and its absorbance was determined at the wavelength of 530 nm.

Cu-Zn Superoxide dismutase measurement: This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The superoxide dismutase (SOD) activity is then measured (at 505 nm) by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay.

RESULTS AND DISCUSSION

In the present study we determined the plasma levels of malondialdehyde, Zinc and erythrocyte Cu-Zn superoxide dismutase in 50 patients with type 2 diabetes mellitus (20 men and 30 women) and 50 control subjects (22 men and 28 women). As shown in Table 2 plasma level of Zn and erythrocyte SOD were significantly decreased and the plasma level of MDA was significantly increased in patients with diabetes compared with controls ($p<0.001$)

Table 2: Plasma malondialdehyde, zinc levels and erythrocyte superoxide dismutase activity

Plasma levels	Patients with diabetes (n = 38)	Controls (n = 19)	p-value
Malondialdehyde (nmol mL^{-1})	6.24±0.85	3.63±0.97	<0.05
Zinc (μg dL^{-1})	116.78±5.51	146.86±9.06	<0.05
Superoxide dismutase (U gr^{-1} Hb)	675.34±60.89	1052.70±52.76	<0.05

The aim of the present study was to determine the plasma level of malondialdehyde, zinc and erythrocyte Cu-Zn supetoxide dismutase enzyme activity in patients with type 2 diabetes. There are a few reports describing difference in plasma lipid peroxidation, zinc and. erythrocyte Cu-Zn supetoxide dismutase enzyme activity between patients with type 2 diabetes versus controls. Some of the studies showed an increase while some other showed a decrease or no significant differences.

Patients with type 2 diabetes mellitus have increased mortality and morbidity compared with controls and are more likely to suffer from coronary artery, cerebrovascular and peripheral vascular diseases (Kannel and McGee, 1979). It has been proposed that oxidative stress may be associated with the pathogenesis of macrovascular complications of type 2 diabetes (Baynes, 1991; Giugliano *et al*., 1995). In people with diabetes, the vulnerability to oxidative damage maybe partly attributed to deficient antioxidant micronutrient status, including trace elements. Impairment of zinc (Chaumer, 1998; Walter *et al*., 1991; Bolstein-Fujii *et al*., 1997; Ruiz *et al*., 1998) status has been reported as an aggravating factor in the progression of diabetes. Although zinc may influence the processes associated with oxidant stress, the practical consequences of its status have not been studied extensively (DiSilvestro, 2000).

The results of this study show that in patients with type 2 diabetes plasma lipid peroxidation (expressed as malondialdehyde) was significantly increased and that zinc levels were decreased compared with controls ($p<0.05$). This study also shows that the erythrocyte superoxide dismutase enzyme activity was decreased in patients with type 2 diabetes compared with controls ($p<0.05$).

Some of the previous studies are in agreement with our findings of increased lipid peroxidation and decreased Cu-Zn superoxide dismutase activity in patients with type 2 diabetes (Vanizor *et al*., 2001; Cho *et al*., 2002). Sundaram *et al*. (1996) studied patients with type 2 diabetes mellitus and showed an increase in lipid peroxidation from the onset of disease. Study of Nourooz-Zadeh *et al*. (1997) showed that changes in lipid peroxidation were related to the underlying metabolic abnormalities in type 2 diabetes rather than to the onset of complications. The some studies describe that plasma

Zn in type 2 diabetes mellitus patients is decreased (Walter *et al*., 1991; Evliaoglu *et al*., 2002), whereas others show no significant differences (Evliaoglu *et al*., 2002; Zargar *et al*., 1998) compared to controls. The results of this study are in agreement with the results of studies showing that plasma Zn levels in patients with type 2 diabetes mellitus is significantly decreased (Walter *et al*., 1991; Evliaoglu *et al*., 2002). But our results are not in agreement with the other studies (Evliaoglu *et al*., 2002; Zargar *et al*., 1998). A possible explanation for this is that there is loss of a large amount of Zn from the body via urine. The source of the Zn that being excreted remains unresolved. There is a concurrent hypozincemia and a decrease in tissue Zn stores. However, it is not clear if this results from hyperzincuria, or from an independent event, an insulin or hyperglycemia related induced loss of Zn from tissue stores. Zn would then be released into the plasma and thereafter excreted. This results in a net loss of total body Zn and eventual hypozincemia.

Possible sources of elevated free radicals in type 2 diabetes include increased production of radical oxygen species, especially from glycation or lipoxidation processes, auto-oxidation of glucose and oxidizing of glucose and decreased antioxidant defense systems (Giugliano *et al*., 1995). Improvement of glycemic control and supplementation with zinc appears to be a beneficial factor in decreasing lipid peroxidation in patients with diabetes. Prevention of lipid peroxidation may help to delay the development of diabetic complications.

The results of this study also show that in type 2 diabetes mellitus patients, erythrocyte Cu-Zn superoxide dismutase activity is decreased. Other studies have reported that erythrocyte superoxide dismutase activity in this type of patients was either decreased (Cigremis *et al*., 2003; Palanduz *et al*., 2001), increased (Turk *et al*., 2002) or no significant differences were observed (Memisogullari *et al*., 2003). Possible explanations for our results include reduced antioxidant protection in type 2 diabetes mellitus and/or greatly increased amounts of free radicals that overwhelm the defense system.

Other explanations include decreased activity of Cu-Zn superoxide dismutase related to either increased free radical production causing oxidation followed by denaturing of the enzyme, or alternatively glycation of the enzyme with resulting inhibition of enzymatic activity (Hunt and Wolff, 1991). Furthermore, zinc is a required cofactor for a variety of antioxidant enzymes, particularly superoxide dismutase. Alterations of zinc metabolism resulting in reduced availability of zinc might be expected contribute to tissue damage observed in diabetes (Sumovski *et al*., 1992).

Increased lipid peroxidation, decreased plasma zinc and reduced erythrocyte Cu-Zn superoxide dismutase activity may predispose patients with type 2 diabetes to cardiovascular complications. These states of affairs may play an important role in progress of Cardiovascular abnormality in patients with type 2 diabetes. We propose that patients with type 2 diadetes may have supraphysiological antioxidants requirements. Supplementation with zinc and other free radical scavengers such as vitamins E and C or foodstuff containing these, such as tomatos, oranges and similars have the potential to boost antioxidant defences and ultimately these important factors up-grade the pateints quality of life and prevent sudden silent myocardial infarction (Dieber-Rotheneder *et al*., 1991; Porkkala-Sarataho *et al*., 1996).

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