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Correlation between oxidative stress and redox-active metals in type I diabetes

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ABSTRACT: Redox-active metals such as copper, iron, zinc and manganese are known to play an important role either as pro- or anti-oxidants in the phenomenon of oxidative stress. In performing their anti-oxidant roles, these metals specifically participate actively via interaction with specific amino acid residues, on the active sites of the antioxidant enzymes superoxide dismutase and catalase. The aim was to explore the nature of the relationship between these metals and oxidative stress levels in type I diabetics. In this work, the serum levels of malondialdehyde (MDA) and the serum concentration of four redox-active metals, zinc, copper, iron and manganese in type I diabetic patients and an in age-matched control group were assayed. The results revealed that MDA concentration was significantly elevated ($p<0.05$) in the diabetic group compared to their control counterparts. Serum iron was also significantly elevated in type I diabetics compared to its level in the control group. Both copper and manganese marginally decreased in type I diabetes relative to the control values. Zinc also decreased in type I diabetic patients compared to the concentration in the control. The Pearson's Product Moment Correlation Coefficient (r) between oxidative stress (MDA) and metals concentrations revealed non-significant linear relationships. This study conclude that these metals do not act individually, but rather in synergy with other cellular factors such as the nature of the amino acids at the active sites of antioxidant metalloenzymes and their protein-bound stores in modulating the oxidative stress level in both normal individuals and Type I diabetes.

Keywords: Oxidative stress, diabetes, superoxide dismutase, catalase, metalloenzymes

INTRODUCTION

A growing body of evidence has indicated that many trace elements play an important role in a number of biological processes by activating or inactivating enzymatic reactions, competing with other elements and metalloproteins for binding sites, affecting the permeability of cell membranes or by other mechanisms (Huang *et al*., 1999). Redox-active metals are members of the transition metals group which posses the capacity to vary their oxidation states. Functionally, redox-active metals play a vital role in the antioxidant defense system. Copper, manganese and iron are known to enable various superoxide dismutases (SOD) as active sites, to carry out their essential role of dismutation, a critical reaction in the antioxidant defense armory. Iron on the other hand is found in the active site of catalase, the enzyme responsible for detoxifying hydrogen peroxide (H_2O_2) arising from cellular metabolism. Hence redox-active metals can be described as active and essential components of the cellular protective mechanism against oxidative and nitrative stress (Rotilio *et al.*, 1995). Under normal conditions the cellular balance between redox-active metal ions is maintained by their sequestration in certain storage forms such as ferritin and transferrin (for iron), ceruloplasmin (for copper) and metallothionein (zinc) among others. However, when an imbalance occurs in the cellular distribution of these metal ions they are known to participate in several reactions that enhance oxidative stress (Fischer and Naughton, 2003). Specifically, by switching oxidation states,

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these metal ions further activate species like hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\cdot-}$) to the highly reactive hydroxyl radical ($OH^{\cdot}$). In addition nitric oxide ($NO^{\cdot}$) reacts with superoxide to form the potent species peroxynitrite ($ONOO^-$) (Fischer and Naughton, 2003; Goetz and Luch, 2008).

Oxidative stress is variously defined as a disruption of redox signaling and control (Dean, 2006) or an imbalance in the biochemical processes leading to production of reactive oxygen species (ROS) and those responsible for the removal of ROS, the so-called cellular antioxidant cascade (Miritim *et al*., 2003). Tissues that become subject to oxidative stress witness steady state levels of ROS-mediated damage to all biomolecules (polynucleotides, proteins, lipids and sugars) leading to a critical failure of biological functions and ultimately cell death (Sayre *et al.,* 2005). Oxidative stress has been suggested to play a role in some physiological conditions and in many disease processes including carcinogenesis, Alzheimer's disease, Parkinsonism, diabetes and metabolic syndrome among others (Huang *et al*., 1999; Castellani *et al.*, 2004; Sayre *et al.,* 2005).

Divalent ions of transition metals can promote lipid peroxidation *in vitro* and much attention is currently focused on lipid peroxidation in the pathogenesis of metal toxicity. The mechanisms of pathogenesis could be mediated by direct effects of certain trace elements e.g. copper or zinc, on the formation of hydroxyl free radicals from hydrogen peroxide and superoxide via Fenton-Haber-Weiss reactions (Huang *et al*., 1999). Several studies have been conducted on both oxidative stress and the role of redox-active metals in diabetes (Baynes and Thorpe, 1999; Hideaki *et al.*, 2005; Mahora *et al.*, 2007; Jensen *et al*., 2009). However from available literature, it is apparent that there still exists a paucity of knowledge on studies on the relationship between the concentration of redox-active metals and the oxidative stress biomarker malondialdehyde.

The present work was undertaken to assess the nature of the association between MDA (an oxidative stress biomarker) and certain redox-active metals in the serum of Type I diabetic patients.

SUBJECTS AND METHODS

Subjects and exclusion criteria

Twenty five adult Type I diabetic patients (mean age, 61.8 years) were involved in this study. There were 25 age-matched healthy volunteers who served as controls. Both patients and controls were recruited from a comparable background (same geographical area and socio-sanitary status). None of the patients or control was placed on hormones, oral contraceptives or trace-metal containing supplements at the time of the study. All were non-smokers. Patients with concomitant diseases such as rheumatoid arthritis, Wilson's disease, cancer or liver diseases were excluded.

Collection of blood sample

Fasting blood samples (5ml) were collected from patients and controls under sterile conditions into metal-free containers by venepuncture. Serum was prepared by centrifuging the blood at 3000 x *g* for 10 minutes at room temperature. The collected sera were stored in metal-free containers at 4ºC until analysis.

Determination of malondialdehyde

Serum malondialdehyde (MDA) was assayed according to the flourometric method described by Wojciech *et al* (1993). Briefly, 50 µL of serum was introduced into 10 mL test tubes containing 1 mL of distilled water. After the addition of the solution of 29 mmol/ of thiobarbituric acid (TBA) in acetic acid (pH of the reaction mixture, 2.4 – 2.6) and mixing, the samples were placed in a water bath and heated for 1 hour at 95-100ºC. Thereafter, the samples were cooled, 25 µL of 5 mol/L of HCl was added (final pH 1.6 – 1.7), and the reaction mixture extracted by agitation for 5 minutes with 3.5 mL of *n*-butanol. The butanol phase was separated by centrifugation at 1500 x *g* for 10minutes. The fluorescence of the butanol extract was determined with a flourometer at wavelengths of 525 nm (excitation) and 547 nm (emission) using a SANCO Fluorescent Photometer – Model 930A.

Determination of metal concentrations

All the glassware used in the determination were first dipped in 5% nitric acid for 48 hours, washed with tap water and deionized water, three times each before drying. Serum samples (0.5 ml) were diluted 10-fold with 0.5% (v/v) solution of Triton-X-100 prior to analysis (Minhe *et al.*, 1989). The concentrations of zinc, copper, iron and manganese were determined in duplicate using a Buck Atomic Absorption Spectrophotometer (AAS), Buck Scientific Instruments, USA.

Statistical analysis

Data was analyzed using Stratigraphics 3.0 Software and Microsoft Excel spreadsheet. Comparison of mean values between controls and Type I diabetics was done using the Students' t-Test. The nature of the relationship between serum malondialdehyde and metal concentration was done using the Product Moment Correlation Coefficient (r). All data were expressed as mean ± SD and were considered significant at $p<0.05$.

Ethics

Ethical approval for this work was obtained from the Ethics Committee of Plateau Hospital Jos, Plateau State, Nigeria. In addition, the guidelines of the Council of International Organization of Medical Sciences / World Health Organization (CIOMS / WHO) (1993) was also adhered to.

RESULTS AND DISCUSSION

Serum malondialdehyde was significantly elevated in Type I diabetics (Table 1) relative to the controls, indicating an elevated level of oxidative stress during this condition. Zinc concentration decreased significantly ($p < 0.05$) in Type I diabetics relative to the concentration of the metal in the controls. With respect to iron, this metal was found to increase in concentration in Type I diabetics when compared to the control values. Marginal, non-significant variations were observed in the concentration of both copper and manganese. The correlation (r) values (Table 2) indicates a much stronger relationship between MDA and zinc and MDA and iron in the controls, and MDA and zinc and MDA and copper in Type I diabetics; even though all the relationships were not statistically significant. However, the relationship between zinc and MDA in control was negative while it was positive in Type I diabetics. This suggests a vital role for zinc in diabetes-induced oxidative stress and anti-oxidant defense.

During diabetes or insulin resistance, the associated hyperglycemia can induce oxidative stress by a number of mechanisms. Specifically, increased levels of glucose reaching the mitochondria leads to an over-drive of the electron transport chain and consequently results in the over-production of superoxide anions which are normally scavenged by mitochondrial superoxide dismutase. The failure of the mitochondrial superoxide dismutase due to excessive reactive oxygen species leads to its conversion to hydroxyl radical and hydrogen peroxide (Nishikawa *et al.*, 2000). In addition the formation of ROS was also increased by free fatty acids through direct effects on the mitochondria (Evans *et al.*, 2002; Du *et al.*, 2000).

Another mechanism whereby high glucose can stimulate oxidative stress is the autooxidation of glucose in the presence of transition metals as well as the generation of ROS during the process of glycation (Wiernsperger, 2003). Indeed, the development of Schiff base via Amadori to advanced glycation end-products (AGEs) is accompanied by ROS-generating reactions at various steps (Yim *et al.*, 2002). Furthermore glucose-derived dicarbonyl compounds react non-enzymatically with the basic amino acids lysine and arginine in proteins to form AGEs both extra and intracellularly. Once formed, AGE-modified proteins cause more oxidative stress. Glycation of proteins in the electron transport chain can impair normal electron flow and promote 'leakage' to form more superoxide radicals.

Table 1: Concentration of malondialdehyde and some redox-active metals of Type I diabetic patients

Parameters	**Control**	**Type I Diabetics**
Malondialdehyde (umol/L)	5.51 ± 0.22^{a}	7.44 ± 0.22^{b}
Zinc (mg/l)	1.28 ± 0.07^{a}	0.79 ± 0.052^{b}
Copper (mg/l)	1.34 ± 0.15^{a}	1.32 ± 0.070^{a}
Iron (mg/l)	1.15 ± 0.08^{a}	1.41 ± 0.09^{b}
Manganese (mg/l)	0.004 ± 0.001^{a}	0.0037 ± 0.0005^{a}

Values with different superscripts between the groups are significantly different ($p<0.05$)

Table 2: Correlation values between malondialdehyde and some redox-active metals of Type I diabetic patients

	Malondialdehyde Versus Redox-Active Metals			
	Zinc	Iron	Copper	Manganese
Control	-0.24	0.31	-0.024	0.06
Type I Diabetics	0.15	0.019	0.27	-0.053

For all correlation values $p > 0.05$

The consequence of non-enzymatic glycation includes alterations of protein structure and molecular surface topology, leading to profound changes in the affected molecule's biochemical properties. Major biochemical effects of excessive glycation include inhibition of regulatory molecule binding, cross-linking of glycated proteins, trapping of soluble proteins by glycated extracellular matrix, decreased susceptibility to proteolysis, inactivation of enzymes and transcription factors, abnormalities of nucleic acid function and increased immunogenicity in relation to immune complex formation (Mohora *et al*., 2007).

Results from the present study has not deviated from the widely established finding that oxidative stress levels are elevated in type I diabetes (Nishikawa *et al.*, 2000; Cerieillo *et al.*, 2001; Mohora *et al.*, 2006; Stephens *et al.*, 2006). The increase in oxidative stress (malondialdehyde) levels in the present study can be attributed to some or all of the aforementioned reasons. It can therefore be hypothesized that even with normal manganese concentration, excessive amounts of superoxide anions reaching the mitochondria can lead to a compromised mitochondrial SOD function leading to the accumulation of H_2O_2 via its conversion from OH^- radical. In addition, the excess iron in type I diabetics can have deleterious implication(s) in the disease. High iron has pro-oxidant activity owing to its ability to participate or catalyze the Fenton / Haber-Weiss reactions, leading to the production of more reactive oxygen species. Excess iron also accelerates the auto-oxidation of glucose and the process of glycation leading to the generation of more reactive oxygen species and increased oxidative stress. Although zinc does not play a catalytic role in the activities of superoxide dismutase, it is nonetheless essential as it ensures that the enzyme and its active site are in the proper structural configuration, requisite for the ROS-scavenging activity of SOD. In this respect, low serum zinc concentration can promote the production of ROS due to compromised Cu/Zn-SOD function associated with SOD conformational deformity. Being a disease with an auto-immune etiology, the enhanced oxidative/respiratory burst of T-cells can also contribute to the elevation in ROS in type I diabetes.

Although the correlation profile did not show a statistically significant, direct linear relationship between oxidative stress (MDA) and redox-active metals

concentration, the shift in correlation values between MDA and redox-active metal ions concentration is suggestive of a derangement in redox-active metal ion homeostasis, which is capable of acting as a contributing factor in favor of enhanced Fenton / Haber-Weiss reactions. In addition, the nature of the non-significant linear relationships existing between oxidative stress (MDA) and the concentration of these redox-active metals raises the possibility that these metals do not act individually, but rather in synergy with other cellular factors such as the nature of the amino acids at the active sites of antioxidant metalloenzymes and their protein-bound stores in modulating the oxidative stress levels in both normal individuals and Type I diabetes.

CONCLUSION

This study has lent evidence to the association between oxidative stress and the pathogenesis of type I diabetes. In particular, it has opened a new path in the understanding of the potential role of redox-active metals, particularly when in excess; in oxidative stress associated disease. Therefore in both the development of novel point-of-care diagnostics and newer approaches to the management of type I diabetes, greater emphasis should be given to addressing the high oxidative stress levels via appropriate anti-oxidant supplementation and the maintenance of a proper redox balance through the monitoring of the concentration of redox-active metals, especially zinc and iron.

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