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Evaluation of plasma superoxide dismutase and glutathione peroxidase in type 2 diabetic patients

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Abstract
Antioxidants are agents that protect, prevent, or reduce the extent of oxidative damage to biomolecules. These agents may be enzymatic, non-enzymatic, or metal chelators. The enzymatic antioxidants include catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx). SOD, a copper, zinc and manganese-containing enzyme, reacts with superoxide radical to form hydrogen peroxide, which is then converted to water by GPx (a glutathione-dependent selenoprotein), or catalase, a heme enzyme. Decreased activity of these antioxidant enzymes may increase the susceptibility of diabetic patients to oxidative injury. An appropriate support of antioxidant supplies may help in preventing clinical complications of diabetes. In view of this, supplementary trace elements such as selenium, copper, zinc, and manganese, essential components of the enzymes, may be useful in preventing the development of diabetic complications. There are number of factors that affect an individual's oxidative status that include gender, age, body composition, smoking status, diet, physical activity level, and the strength of defense mechanism. Hence, this study was carried out to see the relationship of these factors with antioxidant enzymes in clinically diagnosed type 2 diabetes patients.

Keywords: Antioxidant enzymes; superoxide dismutase (SOD); glutathione peroxidase (GPx); type 2 diabetes.

Introduction

Antioxidant enzymes are endogenous proteins that work in combination to protect cells from reactive oxygen species (ROS) damage. Increased levels of the products of oxidative damage to lipids and protein have been detected in the serum of diabetic patients and their presence correlates with the development of complications (Brownlee, 2001). Different studies have provided evidences of increased oxidative stress with depleted antioxidant enzymes and vitamins in both type 1 and type 2 diabetes (Lapolla *et al.*, 2007; Lodovici *et al.*, 2008; Likidlilid *et al.*, 2010; Al-Rawi, 2011). Hyperglycemia, a hallmark of diabetic condition, depletes natural antioxidants and facilitates the production of ROS, which has the ability to react with all biological molecules like lipids, proteins, carbohydrates, DNA and exert cytotoxic effects on cellular components (Dincer *et al.*, 2002). Thus, increased ROS and impaired antioxidant defense contribute for initiation and progression of micro- and macrovascular complications in diabetics (Ceriello *et al.*, 1998; Ceriello and Motz, 2004).

Antioxidants reverse many of the effects of hyperglycemia on endothelial functions such as reduced endothelial dependent relaxation and delayed cell replication (Manisha, 1999). To control lipid peroxidation, there is a defensive system consisting of antioxidant enzymes that play an important role in scavenging ROS. The organisms' susceptibility to free radical stress and peroxidative damage is related to the balance between the free radical load and the adequacy of antioxidant defenses. Abnormally, high levels of lipid peroxidation and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and oxidative stress. Many reports are available with regard to oxidative stress and antioxidant status of type 2 diabetic patients (Giugliano *et al.*, 1995, 1996; Takakura, 1998; Piarulli *et al.*, 2009). Several studies have reported lower concentration of non-enzymatic antioxidants as well as enzymatic antioxidants in type 2 diabetes (Lodovici *et al.*, 2009; Likidlilid *et al.*, 2010; Bigagli *et al.*, 2012), but there is no sufficient data regarding the actual status of antioxidant enzymes in diabetic patients. Therefore, the study was planned to investigate the effects of antioxidant enzymes - superoxide dismutase (SOD) and glutathione peroxidase (GPx) - in type 2 diabetes at various aspects.

Materials and Methods

Patient selection

The study included 120 patients suffering from type 2 diabetes after clinical examination and confirmed diagnosis by physician at ACPM Medical College & Hospital, Dhule (Maharashtra). The basic information of age, occupation, smoking habits, alcohol consumption, duration of disease, family history, medical usage, as well as complications like hypertension was obtained. One hundred and twenty normal non-diabetic healthy persons were carefully selected as controls. They were age and sex matched, not obese, not predisposed, physically active, and their fasting blood glucose level ranged from 70 to 100 mg%. All patients were in the age group of 30–72 yrs of both sexes and were comparable with that of control group. Here, none of the patients and control subjects were taking dietary supplements such as vitamins or minerals. An informed consent from all the participants was obtained before the study.

Sample collection

All the patients and normal healthy individuals (control group) were instructed to fast (overnight) for 12–14 h and then venous blood was collected from the anterior cubital vein with all aseptic precautions in fluoride, plain bulb, and heparinized tubes for biochemical measurements.

Methods

Superoxide dismutase was measured using RANSOD kit and GPx was measured using RANSEL kit (Randox Laboratories Ltd., Crumlin, United Kingdom). This method is based on the study of Paglia and Valentine (1967).

Statistical analysis

Data are expressed as mean ± SD. Statistical significance was evaluated by Student's *t*-test. Differences were considered significant at $p < 0.05$. Correlation between the parameters tested was studied by a regression analysis.

Results

The observations and inference obtained from this study are summarized in Tables 1–3. Among 120 patients, 72 patients are males and 48 are females, and their characteristics are presented in Table 2.

Table 1: Age and sex distribution.

Age (yrs)	Sex		Total	Percentage
	Male	Female		
30–39	16	06	22	18.34
40–49	41	28	69	57.50
≥50	15	14	29	24.16
Total	72	48	120	100.00

Table 2: Characteristics of diabetic patients and controls.

	Type 2 Diabetic Patients (*n* = 120)	Controls (*n* = 120)
Sex (male/female)	72/48	68/52
Age (yrs) (mean ± SD)	44.74 ± 8.95	46.11 ± 8.70
Males	43.45 ± 9.10	46.50 ± 9.09
Females	46.66 ± 8.46	45.61 ± 8.21
Smoking	17/72	—
Alcoholics	8/72	—
Hypertension	46/120	—
FBG (mg/dl)	168.14 ± 62.06*	89.16 ± 17.59
MDA (nmol/ml)	5.06 ± 1.05*	2.69 ± 1.10

*$p < 0.001$ vs. controls.
FBG – Fasting Blood Glucose, MDA - Malondialdehyde

The mean ± SD of age in 120 type 2 diabetic patients is 44.74 ± 8.95 (yrs) and non-diabetic controls is 46.11 ± 8.70 (yrs). Diabetic males were 43.45 ± 9.10 (yrs), diabetic females were 46.66 ± 8.46 (yrs) and mean age of control males was 46.50 ± 9.09 (yrs) and control females was 45.61 ± 8.21 (yrs).

Habits

Out of 72 diabetic males, 17 (23.6%) were smokers and 8 (11.1%) were alcoholic; all the female patients and healthy controls were nonsmokers and non-alcoholics, as shown in Table 2.

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Health status
Among 120 diabetic patients, 46 (38.33%) had the history of hypertension. The fasting blood glucose level of diabetic patients was found significantly very high ($p < 0.001$) compared with non-diabetic healthy controls.

MDA
Mean value of lipid peroxide (MDA) was significantly increased in diabetic group compared with non-diabetics ($p < 0.001$).

Table 3: Antioxidant enzyme levels in diabetic patients and controls.

	n	SOD (U/ml)	GPx (U/ml)
Diabetic patients	120	144.09 ± 33.54*	3792.48 ± 1208.16*
Controls	120	178.65 ± 46.85	4785.65 ± 1217.83
Non-diabetic males	68	190.88 ± 50.21[a]	5277.61 ± 1294.15[aa]
Non-diabetic females	52	162.65 ± 36.73	4142.30 ± 718.07
Diabetic males	72	136.09 ± 32.52[b]	3908.63 ± 1322.41[#]
Diabetic females	48	156.08 ± 31.73	3618.25 ± 1001.14
Non-diabetic age (30–39 yrs)	23	197.82 ± 39.00	4947.39 ± 1582.72
Non-diabetic age (40–49 yrs)	62	180.14 ± 52.79[c]	4809.5 ± 1079.23[#]
Non-diabetic age (≥50 yrs)	35	163.4 ± 34.74	4637.11 ± 1199.54
Diabetic age (30–39 yrs)	23	148.63 ± 20.74	4065.90 ± 711.67
Diabetic age (40–49 yrs)	62	145.28 ± 35.12[d]	3780.55 ± 1318.16[d]
Diabetic age (≥50 yrs)	35	137.79± 37.45	3613.44 ± 1229.50
Uncontrolled DM	53	140.69 ± 35.24[e]	3914.13 ± 1219.36[e]
Controlled DM	67	146.77 ± 32.15	3696.25 ± 1199.61
HT diabetics	46	138.41 ± 41.27[f]	3535.08 ± 1386.74[ff]
Non-HT diabetics	74	147.62 ± 27.41	3952.48 ± 1061.27

Data are presented as mean ± SD.
*$p < 0.001$ vs. controls.
[a]$p < 0.05$ vs. non-diabetic female; [aa]$p < 0.001$ vs. controls.
[b]$p < 0.05$ vs. diabetic female.
[c]$p < 0.05$ vs. non-diabetic age groups between 30–39 yrs, 40–49 yrs, and ≥50 yrs.
[d]$p > 0.05$ vs. diabetic age groups between 30–39 yrs, 40–49 yrs, and ≥50 yrs.
[e]$p > 0.05$ vs. controlled DM.
[f]$p < 0.05$ vs. non-HT diabetics; [ff]$p < 0.001$ vs. non-HT diabetics.
[#]Not significant.
DM – Diabetes mellitus
HT - Hypertension

Discussion

In diabetic patients, the persistence of hyperglycemia has been reported as a cause of increased production of oxygen-free radicals through glucose autoxidation and non-enzymatic glycation. The antioxidant capacity is always decreased in diabetic patients, but it seems necessary to measure all the components to ascertain the reasons. Long-term complications are the main cause of morbidity and mortality (Patel *et al.*, 2008). Although there are some evidences about the role of oxidative stress in the pathogenesis of diabetic complications, the relationship between hyperglycemia and generation of oxidative stress is still unknown. Oxidative stress results from an imbalance between the generation of reactive oxygen and protective mechanisms (West, 2000). Free radicals are the main causes of oxidative stress, which may react with various biomolecules including lipids, carbohydrates, proteins, nucleic acids, and macromolecules of connective tissue (Halliwell, 1994; Rosen *et al.*, 2001; Robertson and Harmon, 2006). Free radicals are difficult to measure directly because of their highly unstable nature, so levels of various lipid peroxidation products have been used as an indicator of free radical activity. Hence, this study was designed to evaluate the activities of these two antioxidant enzymes - SOD and GPx - with various aspects.

At first, we observed that mean value of antioxidant enzymes - SOD and GPx - was significantly decreased in diabetic group compared with non-diabetics ($p < 0.001$). The above results are indicators of decrease in the protective antioxidant mechanism. This is in agreement with several studies, which have been carried out previously. This is because of an increased production of free radicals and ROS. Lipid peroxidation also involves the interaction of free radicals with polyunsaturated fatty acids such as

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arachidonic acid and linolenic acid in membrane lipids. The role of SOD as an antioxidant enzyme consists of dismutation of superoxide anion radical at a rate constant that is 10^4 times the rate constant for spontaneous dismutation at physiological pH.

$$2O_2^{\bullet} + 2H^{\bullet} \xrightarrow{SOD} H_2O_2$$

Superoxide dismutase ensures that no superoxide anion is available to react with hydrogen peroxide to form the hydroxyl radical through the metal-catalyzed Haber–Weiss reaction.

$$O_2^{\bullet} + H_2O_2 \rightarrow O_2 + OH^- + OH^{\bullet}$$

Superoxide dismutase exists in several forms. One form containing manganese is found in the mitochondrial matrix and other containing copper and zinc occurs in the cytoplasm. Cells are capable of increasing synthesis of SOD in response to hyperoxidant stress. Extracellular fluid contains a unique high-molecular weight SOD. The enzyme binds to external endothelial cell surfaces and may be important in the pathogenesis of free radical damage.

This study reveals a significant fall in SOD levels, which could be due to excessive oxidative stress. Decrease in SOD levels can result not only an increase in the superoxide-free radical but also an elevation of other ROS and intensification of lipid peroxidation processes in diabetes. In diabetes, the initial event resulting in the increase in ROS formation is the depletion of adenosine triphosphate (ATP) due to its increased conversion to adenosine monophosphate (AMP), adenosine, inosine, and hypoxanthine. xanthine oxidase, in the presence of oxygen, converts hypoxanthine into xanthine and uric acid accompanied by superoxide formation. Hyperglycemia contributes to oxidative stress by virtue of the fact that monosaccharides and glycolytic intermediates can generate oxidative reactants. Glucose can enolize and thereby reduce molecular oxygen under physiological conditions in the presence of traces of transition metals yielding oxidizing agents like H_2O_2. The glycation reaction itself serves as a source of free radicals. The term autoxidative glycosylation is more appropriate to describe the glucose-dependent oxidative chemical modifications of proteins. Autoxidative glycosylation is initiated by autoxidation of the aldose/ketose sugar to a more reactive dicarbonyl sugar (glucosone), which then reacts with the protein. Partially reduced oxygen intermediates like superoxide anion radical and hydrogen peroxide generated in the course of this autoxidation associated with glycation contribute to the oxidative stress. This is suggestive of the fact that increased autoxidative glycosylation of hemoglobin may also have led to enhanced generation of free radicals like the superoxide anion, thereby causing the depletion of SOD, which quenches it. It can therefore be concluded that hyperglycemia influences the etiopathogenesis of diabetes in more than one way (Tare *et al.*, 1999).

Similarly, in association with selenium, GPx activity would also be expected to be reduced in diabetes. Our data reveal that GPx level was significantly low, indicating decreased scavenging capacity of glutathione-dependent antioxidant defensive system against elevated lipid peroxidation processes in these patients. Glutathione peroxidase is one of the enzymes responsible for the removal of H_2O_2 produced as part of cellular metabolism, and there is possible significance in the occurrence of increased MDA (an indicator of lipid peroxidation) together with reduced levels of GPx in this diabetic group. It is possible that the observed reduction in GPx in these diabetic samples may indirectly lead to increased lipid peroxidation, as lipid hydroperoxides are destroyed by GPx. Another possibility is that erythrocyte GPx activity may in some way be inhibited by the presence of higher levels of MDA (Randox). Similar findings were reported by various authors (Turk *et al*., 2002; Mahboob *et al.*, 2005; Targher *et al.*, 2005; Singhania *et al.*, 2008). Sailaja *et al*. (2003) also reported similar findings. The diabetic adults have shown an increase in MDA level whereas antioxidant activities of enzymes such as SOD, reduced glutathione, and GPx were markedly diminished in comparison to controls.

Effect of gender

This study has examined the relationship between gender and oxidative stress. Mean value of SOD and GPx was higher in non-diabetic healthy male compared with non-diabetic healthy female, and in diabetic condition, these antioxidant levels were slightly decreased but no significant difference was found in diabetic male compared with diabetic female.

This clearly shows that diabetic patients, irrespective of the sex, were exposed to an increased oxidative stress via lipid peroxidation. There are a number of reasons for discrepancies between studies. The external environment is also a significant contributor to oxidative stress. Other factors like body composition, smoking status, diet, physical activity level, and the strength of defense mechanism are also important. The distribution of males and females was more or less uniform with reference to these factors.

Effect of age

There are a number of changes that occur as organisms age. Aging is associated with alterations in body composition, which has implications in the development of insulin resistance and diabetes also. Accordingly, no significant correlation was found between antioxidant enzymes and various age groups in non-diabetic healthy persons. It lowers in diabetic age group ≥50 yrs compared with age groups 30–39 and 40–49 yrs.

These results indicate that oxidative stress impacts the aging process that may be caused by a number of factors including increased free radical production, decreased antioxidant defense system, or a decreased removal or repair. Although the exact mechanisms are not known, it is assumed that oxidative stress is an important factor in the aging process and the individuals who are more resistant to the accumulation of oxidative damage will live longer.

Effect of uncontrolled stage

With regard to controlled and uncontrolled diabetes, SOD and GPx were lower in uncontrolled diabetic patients as compared with controlled diabetic patients. Both these parameters indicated an oxidative stress. The oxidative stress is more during uncontrolled stage of diabetes. Our findings are in accordance with the observations made earlier (Chugh *et al.*, 1999). Chronic exposure to hyperglycemia and insulin resistance has been implicated in altered oxidative metabolism. Excessive plasma and tissue glucose can exert pathological effects through non-enzymatic glycosylation, which leads to the production of superoxide and hydrogen peroxide (Mercuri *et al.*, 2000). A reduced insulin activity and hyperglycemia influence several oxidoreductive pathways including pentose, glycolytic, and sorbitol pathways.

The activities of two major insulin induced enzymes, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, in hexose monophosphate shunt are impaired, leading to reduced NADPH availability. These negatively influence other enzymes and systems involved in defensive processes against oxidative agents, such as the glutathione system, thus increasing oxidative stress. This led us to think that antioxidants might be playing a significant role during uncontrolled stage of diabetes.

Effect of hypertension

Hypertension is a very common problem in patients with diabetes. The prevalence of hypertension in type 2 diabetic patients increases with age. About 35–75% of diabetic complications is related to hypertension. Also, the chance of developing hypertension is doubled in patients with diabetes. Each 10 mmHg rise in systolic blood pressure is associated with a 15% increase in the risk of death due to diabetes (Kirpichnikov and Sowers, 2001). In this view, decreased level of SOD and GPx is observed in diabetic patients with hypertension compared with other diabetic patients. This clearly shows that oxidative stress is more in hypertensive diabetic patients than in only diabetics. Mullan *et al.* (2002) suggest that strict control of blood pressure reduces cardiovascular risk in diabetes, and supplementation of antioxidants like ascorbic acid may potentially be a useful and inexpensive adjunctive therapy.

Conclusion

Alterations in plasma SOD and GPx in type 2 diabetic patients indicate depletion of antioxidant mechanism and prognosis of oxidative stress. Our result shows that gender, age, body composition, smoking status, diet, physical activity level, and the strength of defense mechanisms affect an individual's oxidative status. Therefore, estimations of these antioxidant enzymes might be used as marker in the management of glycemic control and the development of diabetic complications.

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Ethical Approval

The study was approved by ethical committee of ACPM Medical College & Hospital, Dhule (Maharashtra). The patients' consent was taken for the study.

Conflict of Interests

Authors declare that they have no conflict of interests with the publication of this study.

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