

TOTAL ANTIOXIDANT CAPACITY, SUPEROXIDE DISMUTASE AND GLUTATHION PEROXIDASE IN DIABETIC PATIENTS

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SUMMARY : In diabetic patients, the persistence of hyperglycemia has been reported as a cause of increased production of oxygen-free radicals through glucose autoxidation and nonenzymatic glycation. The antioxidant capacity is always decreased in diabetic patients, but it seems necessary to measure all the components to ascertain the reasons. The aim of this study was to determine the plasma total antioxidant capacity (TAC) and changes in the activities of two antioxidant enzymes; superoxide dismutase (SOD) and glutathion peroxidase (GPX) in diabetic patients and to estimate their relationship to levels of glycated hemoglobin, fasting blood sugar and duration of diabetes.

The changes in the status of antioxidant enzymes were evaluated in erythrocyte samples obtained from 125 diabetic patients (types I and II) and 120 apparently healthy sex and age matched subjects as control group. The activities of SOD and GPX were determined by standard spectrophotometric methods. Total antioxidant status was measured using Randox kit. Level of fasting blood glucose was measured by enzymatic method and that of glycated hemoglobin (GHb), by colorimetric method employing thiobarbituric acid reaction.

Serum glucose and GHb levels were high in two types of diabetic patients versus the control group. Compared with the control, the total antioxidant capacity was depleted in two diabetic groups, but depletion was more severe in second type. The activities of SOD and GPX were significantly low in two types of diabetic patients. Marked differences in the activities of the enzymes in good, fair and poor controlled patients were noticed. The enzyme activities in first type were higher than that of type II, but the differences were not significant. In diabetic patients, significant correlation between the total antioxidant capacity and levels of GHb, fasting blood sugar and duration of diabetes was observed, but in the case of SOD and GPX it was not marked.

In view of low activities of the enzymes in both types of diabetic patients and lack of correlation between their enzymes activities, levels of glycated hemoglobin, fasting blood sugar and duration of disease it may be concluded that reduction in the activities of the enzymes are partially involved in depletion of the total antioxidant capacity. It seems that the reduction in levels of other antioxidant enzymes and substances are involved in the decreased antioxidant capacity in diabetic patients. In view of low activities of SOD and GPX in patients supplementary trace elements such as Selenium, Copper, Zinc and Manganese, the essential components of the enzymes structures may be useful in prevention of oxidative stress. The meaningful correlation between depletion of total antioxidant capacity and poor glycemic control suggests that measurement of total antioxidant capacity in diabetic patients can be used as an index of glycemic control and development of diabetic complications in both types of diabetes.

Key Words: Diabetes, Antioxidant enzymes, superoxide dismutase (SOD), glutathion peroxidase (GPX).

INTRODUCTION

It is now well recognized that diabetes is an epidemic disease in most countries that are undergoing socio-economic transitions. World wide, an estimated 150 million people are affected by diabetes, and this number is likely to reach 300 million by the year 2025 if successful strategies are not implemented for its prevention and control (1). Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and the most common complications such as atherosclerosis, nerve damage, renal failure, male impotence and infection (2). Recently, some evidences suggest that oxidative stress may play an important role in the etiology of diabetes and diabetic complications (3). In healthy individuals, oxidative damage to tissue is prevented by a system of defenses which includes antioxidant enzymes and small molecules with scavenging ability such as antioxidant vitamins (4). In diabetic patients an altered balance between reactive oxygen species production and antioxidant levels has been reported (5,6) but there is still lack of data regarding the actual status of antioxidant enzymes in diabetic patients. In order to gain more information about the activities of antioxidant enzymes, in this study SOD, GPX and also total antioxidant capacity (TAC) were estimated in patients suffering from diabetes mellitus. The correlation between the enzymes activities, total antioxidant status glycated hemoglobin, fasting blood sugar (FBS), duration of diabetes and type of diabetes were determined.

MATERIALS AND METHODS

Subjects

The study included 75 patients suffering from second type of diabetes (38 males and 34 females) aged between 35 and 66 years and 30 patients with type I diabetes (17 males and 13 females) aged between 9 and 45 years. Two separate control groups, one for type II (65) and other for type I (30), comparable with respect to sex and age of the diabetic patients were selected.

Sample preparation

Venous blood samples (8 ml) were obtained from all patients and controls after overnight fast. About 2 ml of the sample was collected in heparinized tubes, used for determination of GPX activity in erythrocytes and plasma levels of total antioxidant and glucose. The rest of sample (about 6 ml) was collected in tube

containing EDTA and used for determination of erythrocytes SOD and GHb.

Methods

SOD activity was measured in erythrocyte samples using commercially available kit (Ransel; Randox Laboratories Crumlin U.K.). The hemoglobin concentration in milligrams per millilitres was determined by cyanmethinoglobin method (7). Erythrocyte GPX activity was determined using a commercial kit (Ransel; Randox) and expressed as units per gram of hemoglobin. This method is base on Paglia and Valentine (8). Plasma total antioxidant capacity was measured using Randox total antioxidant status kit in which ABTS (2, 2-Azino-di [3- ethyl benzthiazolin sulphamate]) is incubated with a peroxidase and H₂O₂ to produce the radical cation ABTS⁺. This has a stable blue green color, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree which is proportional to their concentration (9).

Statistical analysis

Data are expressed as mean \pm 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 clinical characteristics and laboratory data of patients and control subjects are summarized in Table 1.

According to GHb levels, the diabetic patients were divided into three groups, patients with good diabetic control (GDC) with GHb level less than 9.0%, patients with fair diabetic control (FDC) with GHb level between 9.0%-11.0% and patients with poor diabetic control (PDC) with GHb level more than 11.0% (Table 2).

As expected, there are significant differences in mean level of GHb among three groups of both types of patients ($p < 0.01$ in all cases).

The activities of SOD and GPX in patients and control were determined. As shown in Table 3, significant reduction in the activities of both enzymes in both types of patients was noticed ($p < 0.001$).

The SOD and GPX activities in first type were higher than that of type II, but the differences were not significant. However, statistical analysis (Anova) showed that differences in the mean levels of activities of two antioxidant enzymes among three groups of diabetic patients in both types of diseases were not significant ($p > 0.1$ in all cases).

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Table 1: Clinical characteristics and laboratory data of patients and control subjects.

Clinical and laboratory data	Patients			
	Type I	Control	Type II	Control
Number	30	30	75	65
Sex (M/F)	17/13	20/10	38/37	40/25
Age (year)	25 ± 11	26 ± 13	50 ± 8	52 ± 10
Duration of diabetes	12 ± 9.8	0	6.75 ± 4	0
FBS (mg/dl)	136 ± 6	79 ± 5	69.8 ± 41	88.3 ± 9
Hb (g/dl)	13.60 ± 2	14.10 ± 2.1	13.95 ± 1.4	14.30 ± 1.3
GHb (%)	9.1 ± 1.65	4.1 ± 1.21	10.12 ± 1.95	5 ± 0.65

Compared with the control group, the total antioxidant capacity (TAC) was depleted in two diabetic groups, but the depletion was more severe in patients with type II. As shown in Table 4, differences in the depletion of TAC in three subgroups of both types was statistically significant ($p < 0.001$).

Table 2: Frequency distribution of patients according to GHb levels.

Groups	Type I		Type II	
	Number	GHb (%)	Number	GHb (%)
GDC	17	7.30 ± 0.5	26	8 ± 0.7
FDC	8	9.85 ± 4.9	23	9.77 ± 0.66
PDC	5	12.75 ± 0.7	0.26	12.99 ± 0.85

In both groups of diabetic patients possible correlation between the activities of antioxidant enzymes, SOD and GPX and total antioxidant capacity with levels of GHb and FBS, duration of diabetes and age were also studied. There were no significant correlations between the enzymes with each of the above mentioned clinical characteristics in both types of diabetics patients, but in the

case of serum total antioxidant capacity (TAC) correlation was marked. The statistically meaningful results are shown in Table 5

DISCUSSION

Diabetes mellitus is a complex syndrome affecting 3% to 5% of people (10). Long-term complications are main cause of morbidity and mortality (11). While there are some evidences about the role of oxidative stress in the pathogenesis of diabetic complications, but relationship between the hyperglycemia and generation of oxidative stress is still unknown. Oxidative stress results from an imbalance between the generation of reactive oxygen and protective mechanisms (12). Free radicals, the main causes of oxidative stress, may react with variety of bio-molecules including lipids, carbohydrates, proteins, nucleic acids and macromolecules of connective tissue. The oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases (13).

The present study was designed to evaluate the activities of two antioxidant enzymes; SOD and GPX and total antioxidant capacity in diabetic patients and to examine the relationship between the measured parameters,

Table 3: Mean activity of SOD and GPX in patients and control.

Variables	Type I		Type II	
	Patient	Control	Patient	Control
SOD (U/g Hb)	543 ± 52	902 ± 169	490 ± 31.5	883 ± 173
GPX (U/g Hb)	31 ± 6.2	38 ± 6.5	29.8 ± 4.3	35 ± 5

Table 4: Total antioxidant capacity (TAC) in patients with types I and II diabetes and their subgroups.

Groups and Subgroups	Total Antioxidant Capacity (TAC) mmol/l Plasma in:	
	Type I	Type II
Control	2.5 ± 0.45	2.1 ± 0.5
Total Patients	1.7 ± 0.5	1.4 ± 0.45
Good Diabetic Control (GDC)	2.3 ± 0.3	1.8 ± 0.55
Fair Diabetic Control (FDC)	1.8 ± 0.25	1.6 ± 0.4
Poor Diabetic Control (PDC)	1.40 ± 0.35	1.23 ± 0.12

glycemic control, duration of diabetes and age of the patients.

In the present study, the activities of two antioxidant enzymes; SOD and GPX in erythrocytes of patients suffering from first and second types of diabetes mellitus were significantly lower than those of control groups. The lowest activities were noticed in patients with poor diabetic control. These findings agree with a number of studies (14) and are not compatible with others (15). In diabetic patients, the autoxidation of glucose results in the formation of hydrogen peroxide which inactivates SOD (16). Therefore, the accumulation of hydrogen peroxide may be one of the explanations for decreased activity of SOD in these patients. The primary catalytic cellular defense that protects cells and tissues against potentially destructive reactions of superoxide radicals and their derivatives is the Cu/Zn-SOD. It has been observed that SOD can be rapidly induced in some conditions when cells or organisms are exposed to oxidative stress (17). The highest SOD activity

in red blood cells at the onset of diabetes and subsequent decrease in its activity have been reported (18). The low activity of SOD in our study in both types of diabetes may suggest that with longer disease duration, SOD induction and consequently its activity progressively decrease, since nonenzymatic glycation, the other cause of hydrogen peroxide production, later predominates and further inhibition of Cu/Zn SOD occurs.

Our results showed that erythrocyte GPX activity was significantly lower in patients suffering from first and second types of diabetes compared with control subjects. The results agree with those of Jos *et. al.* (19) who reported decreased GPX in group of first type of diabetic adolescents with retinopathy. However, some authors found no differences between GPX activity of types I or II diabetic patients and control (19, 21). The low activity of GPX could be directly explained by the low content of GSH found in diabetic patients, since GSH is a substrate and cofactor of GPX (18). Enzyme inactivation could also contribute to low GPX activity. GPX is a relatively stable enzyme, but it may be inactivated under conditions of severe oxidative stress (22). Inactivation of the enzyme may occur through glycation governed by prevailing glucose concentration (23). Thus increased glycation in diabetic patients and subsequent reactions of proteins may affect amino acids close to the active sites of the enzyme or disturb the stereochemical configuration and causes structural and functional changes in the molecule.

The low activity of GPX causes accumulation of H₂O₂ in diabetic patients. This finding could also explain the progressive decrease in SOD in later stages of the diabetes.

Total antioxidant status or total antioxidant capacity within a matrix such as food extract, beverage or body fluid (plasma, synovial fluid etc.) should reflect the collective contribution to reducing properties of the individual antiox-

Table 5: Correlation between total antioxidant capacity (TAC) and clinical characteristics of patients.

Clinical and biochemical characteristics	TAC (mmol/l) in Type I		TAC (mmol/l) in Type II	
	r	p	r	p
GHb (%)	- 0.365	0.002	- 0.398	0.0015
FBS (mg/dl)	- 0.293	0.03	- 0.325	0.021
Duration of diabetes (year)	0.310	0.035	0.334	0.015
Age (year)	0.278	0.04	0.355	0.004

idants or electron-donating components. Thus, in some instances, total antioxidant potential might indeed be more informative than knowledge of the levels of individual antioxidant constituents per se. In this study, depletion of total antioxidant capacity estimated in first and second types of diabetic patients. Compared with the control groups the total antioxidant capacity was depleted in both types of diabetes. The depletion was more severe in patients with type II diabetes. Significant correlation between the antioxidant capacity and glycemic control of the patients was noticed. The depletion of total antioxidant capacity in second type of diabetes has been reported by others (24). Reduced total antioxidant capacity in first type of diabetes and its association with coronary artery calcification has also been indicated (25). In our study the depletion of antioxidant capacity in second type of diabetic patients was higher than that in first one. The finding may be due to poorer glycemic control of type II diabetic patients than that of type I.

In spite of low activities of SOD and GPX in both types of diabetes, a negative, but not significant correlation between the activities of the enzymes and levels of glycated hemoglobin, fasting blood glucose and duration of diabetes was observed. The findings are compatible with those of reported by Ruiz *et. al.* (26) but not with those of Domingues *et. al.* (18). To gain more information about antioxidant status in diabetic patients the correlation between total antioxidant capacity and clinical characteristics of the patients was estimated. The observed marked correlation between the total antioxidant capacity and all the variable in both groups of the patients confirms the reported results of other workers (24).

Decreased activity of the antioxidant enzymes and depletion of total antioxidant capacity may increase the susceptibility of diabetic patients to oxidative injury. Appropriate support for enhancing antioxidant supplies may help to prevent clinical complications of diabetes mellitus. In view of low activities of the SOD and GPX in diabetic patients it was concluded that supplementary trace elements such as Selenium, Copper, Zinc and Manganese, the essential components of the enzymes structures may be useful in preventing the development of diabetic complications. The significant correlation between total antioxidant capacity and clinical characteristics of diabetic patients including their blood levels of glucose and gly-

cated hemoglobin suggests that the measurement of total antioxidant capacity in diabetic patients can be a marker of glycemic control.

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