

<sup>1</sup> Department of Medicine, Dr Josip Benčević General Hospital, Slavonski Brod, Croatia

<sup>2</sup> University Department of Medicine, Dubrava University Hospital, Zagreb, Croatia

<sup>3</sup> Andrija Štampar School of Public Health, Zagreb, Croatia

<sup>4</sup> Vuk Vrhovac Institute, University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Zagreb, Croatia

<sup>5</sup> Department of Gynecology and Obstetrics, Dr Josip Benčević General Hospital, Slavonski Brod, Croatia

## IMPACT OF GLYCEMIC CONTROL ON ANTIOXIDANT ENZYME ACTIVITY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

Marica Jandrić-Balen<sup>1</sup>, Veljko Božikov<sup>2</sup>, Jadranka Božikov<sup>3</sup>, Željko Metelko<sup>4</sup>, Ivan Jandrić<sup>5</sup>, Željko Romić<sup>2</sup>

*Key words: diabetes mellitus, antioxidant enzymes, glyceemic control*

### SUMMARY

*The study evaluated serum activity of the key antioxidant enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase (GLPX) as well as total antioxidant status (TAS) in diabetic and nondiabetic patients, and investigated the correlation between serum concentration of glycated hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) as a parameter of glyceemic control and antioxidant enzyme activity. The study included 211 subjects, 109 of them patients with type 2 diabetes mellitus. All study subjects were examined and standardized according to essential parameters including laboratory assessment of serum HbA<sub>1c</sub> concentration.*

*The key antioxidant enzyme activity and TAS were assessed in serum samples using colorimetric Randox kits. Diabetic patients had significantly lower activities of catalase, GLPX and TAS ( $p=0.008$ ,  $p<0.001$  and  $p=0.001$ , respectively) and significantly increased SOD activity ( $p<0.001$ ). There was no significant correlation of serum HbA<sub>1c</sub> concentration with either key enzyme activities or TAS. The study demonstrated the presence of oxidative stress in diabetic patients, which was not significantly influenced by glyceemic control.*

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Correspondence to: Marica Jandrić-Balen, MD, Department of Internal Medicine, Dr Josip Benčević General Hospital, Andrije Štampara 42, HR-35000 Slavonski Brod, Croatia  
E-mail: mjandricbalen@yahoo.com

### INTRODUCTION

It is well known that oxidative stress plays a major role in the pathogenesis of many diseases, and a defective natural antioxidant status was observed in many of these diseases (1). According to Helmut Sies, oxidative stress is defined as a shift in balance in cellular oxidation-reduction reactions in favor of oxidation, which leads to damage to the cell and formation of molecular products that are indicators of oxidative stress (2). During their evolution, aerobic organisms have developed protection and defense mechanisms against oxidants and free radicals. The antioxidant system includes numerous enzyme and nonenzyme type of antioxidant groups that are located in the cell and in the extracellular fluid. Antioxidant is a substance that protects the biological tissue from damage due to free radicals, and can be recycled or regenerated by biological reducers (3). Antioxidant system is an integrated defense network with many different mechanisms for protection and repair caused by oxidative damage. Enzymes have the key role in this defense from oxidative stress. The three most important antioxidant enzymes are superoxide dismutase (SOD), catalase, and glutathione peroxidase (GLPX) (2). Total plasma antioxidant status (TAS) represents the level of cumulative antioxidant reserve of the body and enables evaluation of the average antioxidant potential.

Diabetes mellitus (DM) is a state of increased oxidative stress, based on increased peroxidation and reduced antioxidant status (4,5). It is noted that prolonged hyperglycemia, due to its ability of nonenzymatic protein glycation, may alter cellular functions and cause oxidative damage to the cellular membrane (6-10). Auto-oxidation processes, present in DM, give rise to the formation of free radicals, which cause damage either directly affecting a specific molecule or indirectly by forming numerous toxic derivatives (6,10-12). On the other hand, many prospective studies have confirmed that serum hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) concentration is an important predictor of both macrovascular and microvascular complications of DM including coronary mortality and lower extremity amputations (13).

## SUBJECTS AND METHODS

A group of 109 patients with type 2 DM and 102 nondiabetic control subjects were included in the study. All study subjects were assessed at the County Center for Diabetes, Slavonski Brod, and signed the Informed Consent Form prior to enrolment in the study. All study subjects were examined and standardized according to the essential parameters of age, sex, body mass index (BMI), and duration and treatment of DM. Using standard laboratory procedures, fasting plasma glucose concentration and serum concentration of HbA<sub>1c</sub> were determined. The key antioxidant enzymes, SOD, catalase and GLPX as well as TAS activity were assessed in serum samples using colorimetric Randox kits (Randox Laboratories, Ltd., United Kingdom).

Statistical analysis was done on a computer using the Statistica for Windows, ver. 5.5. software (Tulsa, StatSoft, Inc., 2000). Testing of three numerical characteristics was done by use of Mann-Whitney test, and testing of correlation of two numerical characteristics by use of Spearman's test.

## RESULTS

The mean age was 60 years for both diabetic and nondiabetic groups. Diabetic group consisted of 57 (52%) male and 52 (48%) female patients. The mean BMI in diabetic and nondiabetic group was 26.46 kg/m<sup>2</sup> and 26.00 kg/m<sup>2</sup> with SD of 3.69 kg/m<sup>2</sup> and 3.77 kg/m<sup>2</sup>,

Table 1. Mean value and standard deviation of antioxidant enzyme serum concentrations in diabetic and nondiabetic patients

	Diabetic patients (n=109)		Nondiabetic patients (n=102)	
	χ	SD	χ	SD
Total antioxidant status (mmol/L)	1.61	0.07	1.69	0.09
Superoxide dismutase (IU/L)	30.49	2.98	29.01	2.15
Catalase (kIU/L)	78.60	13.10	81.49	13.33
Glutathione peroxidase (IU/mL)	236.14	28.35	254.20	24.92

Table 2. Difference in antioxidant enzyme activity and total antioxidant status between diabetic and nondiabetic patients (Mann-Whitney test)

Total antioxidant status			
Average rank	N		
83.06	109	Diabetic patients	
130.51	102	Non-diabetic patients	
	211	Total	
U		Z	p
3,058.5		-5.648	0.000
Superoxide dismutase			
Average rank	N		
122.56	109	Diabetic patients	
88.31	102	Non-diabetic patients	
	211	Total	
U		Z	p
3,754.5		-4.112	0.000
Catalase			
Average rank	N		
95.22	109	Diabetic patients	
117.52	102	Non-diabetic patients	
	211	Total	
U		Z	p
4,384.0		-2.651	0.008
Glutathione peroxidase			
Average rank	N		
84.29	109	Diabetic patients	
129.20	102	Non-diabetic patients	
	211	Total	
U		Z	p
3,192.5		-5.341	0.000

respectively. Among diabetic patients, 56 (51%) were insulin treated patients, and the rest were treated with oral hypoglycemic drugs. Also, 72 (66%) diabetics had a DM duration of over 5 years, and the rest had a shorter

Table 3. Correlation of antioxidant enzyme activity and total antioxidant status with serum concentration of HbA<sub>1c</sub> ( $\chi=8.35\%$ ,  $SD=1.92\%$ ) in diabetic patients (n=109) (Spearman's test)

		Catalase	Superoxide dismutase	Total antioxidant status	Glutathione peroxidase
HbA <sub>1c</sub>	Coefficient of correlation	0.026	-0.024	-0.023	-0.051
	p	0.788	0.802	0.812	0.598

DM duration. The mean fasting glucose was 11.83 mmol/L (SD 3.52 mmol/L) in diabetic group, and 6.61 mmol/L (SD 0.88 mmol/L) in nondiabetic group.

The mean values and standard deviations of serum antioxidant enzyme concentrations measured in diabetic and nondiabetic groups are shown in Table 1. Table 2 shows results of testing the difference in antioxidant enzyme activity between diabetic and nondiabetic patients using Mann-Whitney test. A statistically significant difference between diabetic and nondiabetic patient groups was found for all four parameters tested.

In diabetic patients, the mean value of the serum HbA<sub>1c</sub> concentration was 8.35% (SD 1.92%). Results of Spearman's rank correlation test of antioxidant enzyme activity and TAS with serum HbA<sub>1c</sub> yielded no correlation between serum HbA<sub>1c</sub> concentration and antioxidant status in the group of diabetic patients (Table 3).

## DISCUSSION

Many studies have demonstrated the presence of oxidative stress in DM as an expression of increased free radical production and diminished antioxidant defense (4-6,14,15). Also, there are many different markers that can be used to prove the presence of oxidative stress in DM (16). Free radicals are very unstable due to their high reactivity (5,17). Because of their nature, they have a short lifetime and are difficult to measure and accurately determine *in vivo* as well as in biological material such as plasma or other body fluids (18-20). In clinical states, their existence is determined by their influence on other molecules or antioxidant mechanisms which they cause (20), so it is more reliable to measure the consequences of their action, mostly a decreased level of antioxidant enzyme activity (21).

We evaluated antioxidant status through the activity of the key antioxidant enzymes in patients with type 2 DM and in a healthy control group. Results of our study indicated impaired antioxidant status in diabetic patients as a sign of the presence of oxidative stress. We found a statistically significant decrease in the catalase and GLPX activity as well as lowered TAS in diabetic patients, whereas SOD activity was significantly increased in comparison with the healthy control group. These findings are generally consistent with literature data and are a clear indicator of the presence of oxidative stress in DM (22-25).

In our study, we also focused on the correlation between HbA<sub>1c</sub> concentration and antioxidant enzyme activity. We could not confirm any change in the antioxidant status as a result of different glyceemic control level. Similar results on the absence of glyceemic control (and even of DM duration) impact have been reported by other authors (26,27). Japanese authors, however, have demonstrated a proportional decrease in the antioxidant enzyme activity with declining glyceemic control adequacy (28). Such contradictory findings could be explained as a consequence of insufficient standardization of clinical and/or analytical procedures utilized in studying the issue. Recent studies on hemoglobin glycation and erythrocyte SOD inactivation *in vitro* and *in vivo* also failed to confirm any significant correlation between SOD activity and nonenzymatically glycated hemoglobin concentration (29). This observation may be due to a longer half-life of hemoglobin in comparison to SOD, so evaluating glyceemic control for a period of previous 3 months using glycated hemoglobin concentration cannot accurately reflect the impact of glyceemic control on SOD activity.

In conclusion, our study demonstrated the presence of oxidative stress in diabetic patients, which was not significantly influenced by glyceemic control, although this parameter has been recognized as a predictor of the onset and development of diabetic vascular complications. As oxidative stress had also been

implicated in the pathogenesis of diabetic complications (8,12,14,28,30-32), many questions remain to be answered concerning free radicals and antioxidants, especially their role in DM.

Future investigations of free radicals and antioxidant defense mechanisms should clarify the role of free radicals in the etiology and pathogenesis of DM, whereas "antioxidant approach" should be considered for future prevention and therapy strategies.

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