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Antioxidant Enzymes Activity in Patients with Peripheral Vascular Disease, with and without Presence of Diabetes Mellitus

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A B S T R A C T

The study evaluated antioxidant status in patients with peripheral vascular disease (PVD), with and without concomitant diabetes mellitus (DM). 211 participants were divided into standardized 4 groups: patients with PVD and DM (PVD+DM+), patients with PVD without DM (PVD+DM-), patients without PVD with DM (PVD-DM+) and patients without PVD and DM (PVD-DM-). The diagnosis of PVD was established by Doppler sonography analysis, including determination of the ankle brachial index (ABI), partial pressures along the leg, and CW Doppler sonography at typical locations. Antioxidant status has been evaluated through the colorimetrically assessed serum activity of key antioxidant enzymes: superoxide dismutase (SOD), catalase, and glutathione peroxidase (GLPX) as well as through total antioxidant status (TAS) determination. In PVD+DM- group, as well as PVD-DM+ group, a significantly lower activity of the GLPX, catalase and TAS was found, whereas activity of SOD was significantly higher. There was no statistically significant difference between PVD+DM+ and PVD-DM+ group. Likewise, there was no statistically significant difference between PVD+DM- and PVD -DM-group. This study has shown that there is statistically significant difference in activity of antioxidant enzymes between diabetic and non-diabetic patients, irrespectively of PVD presence. Furthermore, PVD present alone does not alter key antioxidant enzymes activity in comparison with healthy subjects.

***Key words**: peripheral vascular disease, diabetes mellitus, antioxidant enzymes*

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Introduction

Oxidative stress is defined as a shift in balance in cellular oxidation-reduction reactions in favor of oxidation. This leads to damage of the cell and formation of molecular products that are indicators of oxidative stress[1]. The rise in oxidative stress can be caused by hyper production of reactive forerunners of oxygen radicals or due to the decrease in activity of scavengers in the defense antioxidant system. Oxidative stress develops when an increased production of free radicals exceeds anti-oxidation defense, which then results in tissue damage[2].

Aerobic organisms have developed protection and defense mechanisms against oxidants and free radicals during their evolution. Antioxidant is a substance that protects the biological tissue from damage due to free radicals, and can be recycled or regenerated by biological reducers[2]. The system includes numerous enzyme and non-enzyme type of antioxidant groups that are located in the cell and in the extracellular fluid.

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)[1].

Chronic complications of diabetes mellitus (DM) represent the main cause of infection and death in diabetic patients[3,4] even in the absence of hypertension, smoking and hyperlipidemia[5]. Those complications are heterogeneous groups of clinical disturbances that attack the vascular system, kidneys, retina, peripheral nerves, lens, skin, and present the main threat to the quality and length of life in diabetic patients[4].

DM represents the state of increased oxidative stress, based on increased peroxidation and reduced antioxidant status[6]. Oxidative stress is also a recognized pathogenic mechanism in diabetic complications[7].

The goal of this study is to analyze the antioxidant status in patients with peripheral vascular disease (PVD), both those with and without presence of DM.

Subjects and Methods

The study includes 4 groups of participants whose antioxidant status has been determined:

I. composed of 53 participants with PVD and with DM (PVD+ DM+);
II. composed of 59 participants with PVD and without DM (PVD+ DM-);
III. composed of 56 participants without PVD and with DM (PVD- DM+);
IV. composed of 43 participants without PVD and without DM (PVD- DM-).

All study groups were standardized according to parameters included in individual patient index card such as age, sex, body mass index (BMI), type and duration of DM, anti-diabetic treatment and also laboratory findings about fasting plasma glucose concentration and HbA_{1C}.

Assessment of macrocirculation of lower extremities was performed using the standard Doppler sonography analysis, which includes the CW Doppler sonography at typical locations, determining the ankle brachial index (ABI), and measuring partial pressures at four levels along the leg. CW Doppler sonography device, Vasoscan VL from a British company Oxford/Sonicaid, was used for this purpose.

Diagnosis of PVD is established for ABI values less than 0.9. Isolated partial disease has ABI values greater than 0.5. ABI values smaller than 0.5 indicate multisegmental obliterative disease. Systolic partial pressures that are determined at four levels along the leg are used in estimating stenosis localization, and

all are usually greater than the brachial pressure. The allowable vertical pressure gradient between adjacent segments does not exceed 30 mm Hg.

Serum samples obtained for the purpose of the study were frozen immediately. Measuring the serum activity of key antioxidant enzymes: SOD, catalase and GLPX, as well as TAS was done by using colorimetrical Randox kits (Randox Laboratories Ltd, United Kingdom) and expressed in IU/l, kIU/l, IU/ml and mmol/l, respectively.

Statistical analysis was done on a computer using the software STATISTICA for Windows, ver. 5.5., Tulsa, StatSoft, Inc. 2000. Testing the difference between groups utilized the Kruskal-Wallis. Testing three or more numerical characteristics was done by using the Mann-Whitney test.

Results

Figure 1 shows the distribution of serum GLPX activity for four observed groups. Using the Kruskal-Wallis test, statistically significant difference has been shown between groups ($p<0.001$). GLPX activity is significantly lower in diabetic patients than in groups without DM.

Figure 2 shows the distribution of serum catalase activity for four observed groups. Statistically significant difference has been shown between groups ($p=0.003$). Catalase activity is significantly lower in PVD-DM+ group, while non-diabetic patients have higher catalase activity.

Figure 3 shows the distribution of TAS activity in serum for four observed groups. Statistically significant difference has been shown between groups ($p<0.001$). TAS activity is significantly lower in diabetic patients than in groups without DM.

Figure 4 shows the distribution of serum SOD activity for four observed groups. Using the Kruskal-Wallis test, statistically significant difference has been shown between groups ($p<0.001$). SOD activity

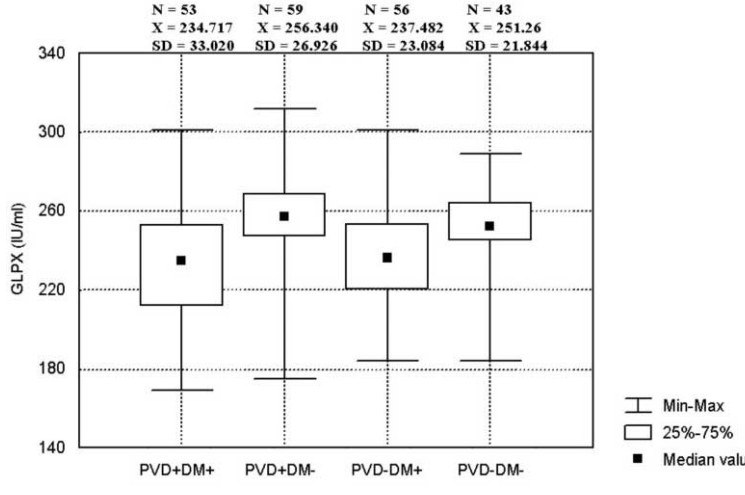

Fig. 1. Distribution of serum glutathione peroxidase (GLPX) activity for four observed subject groups: with PVD and with DM (PVD+DM+), with PVD and without DM (PVD+DM-), without PVD and with DM (PVD-DM+), and without PVD and without DM (PVD-DM-), (PVD = peripheral vascular disease, DM = diabetes mellitus).

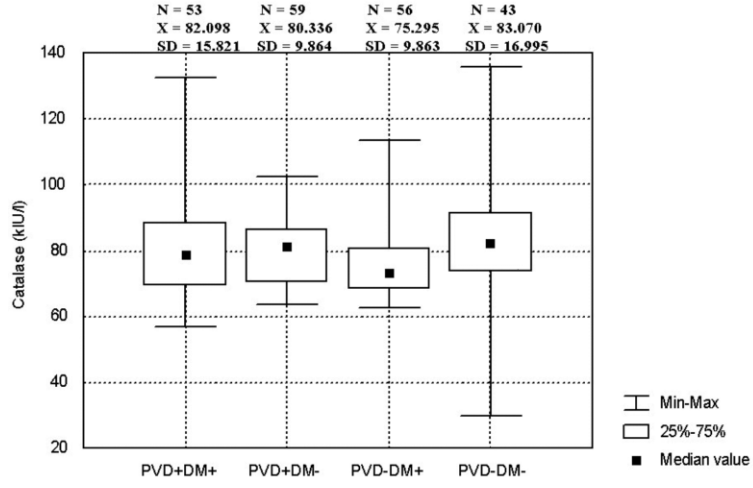

Fig. 2. Distribution of serum catalase activity for four observed subject groups: with PVD and with DM (PVD+DM+), with PVD and without DM (PVD+DM-), without PVD and with DM (PVD-DM+), and without PVD and without DM (PVD-DM-), (PVD = peripheral vascular disease, DM = diabetes mellitus).

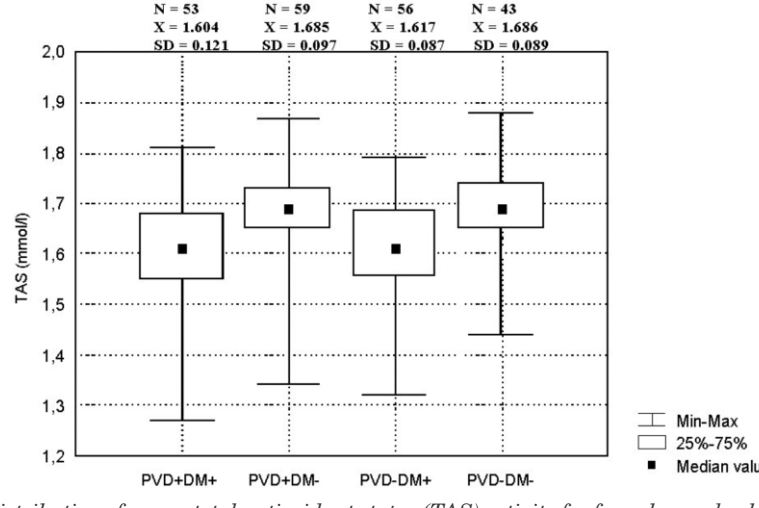

Fig. 3. Distribution of serum total antioxidant status (TAS) activity for four observed subject groups: with PVD and with DM (PVD+DM+), with PVD and without DM (PVD+DM-), without PVD and with DM (PVD-DM+), and without PVD and without DM (PVD-DM-), (PVD = peripheral vascular disease, DM = diabetes mellitus).

is significantly higher in diabetic patients than in groups without DM.

Table 1 shows the results of testing the difference in antioxidant enzyme ac-

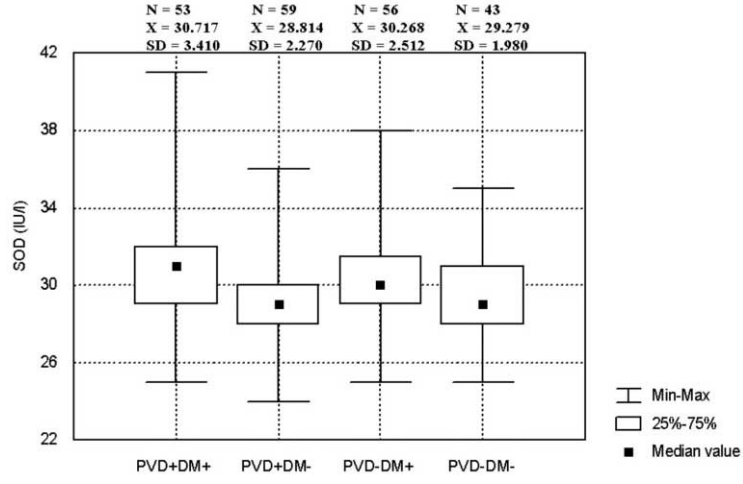

Fig. 4. Distribution of serum superoxide dismutaze (SOD) activity for four observed subject groups: with PVD and with DM (PVD+DM+), with PVD and without DM (PVD+DM-), without PVD and with DM (PVD-DM+), and without PVD and without DM (PVD-DM-), (PVD = peripheral vascular disease, DM = diabetes mellitus).

tivity between diabetic and non-diabetic patients using the Mann-Whitney test. Statistically significant difference has been found between diabetic and non-diabetic patients for all four assessed parameters.

Table 2 shows the results of testing the difference in antioxidant enzyme activity between patients with and without PVD, only in groups with DM, using the Mann-Whitney test. Statistically significant difference has been found between PVD+ and PVD- patients inside the diabetic group for catalase enzyme. There was no significant difference for the remaining three enzymes.

Table 3 shows the results of testing the difference in antioxidant enzyme activity between patients with and without PVD, only in the non-diabetic group and the healthy group, using the Mann-Whitney test. There was no significant difference between PVD+ and PVD- patients inside the non-diabetic group and the healthy group.

Discussion

Vascular complications as consequences from atherosclerosis are the main causes for infection and death in DM. Diabetic patients suffer more instances of coronary disease, stroke, and PVD even without other risk factors[8]. In recent years, there have been many discussions about the possible role of oxidative stress in pathogenesis and progression of diabetic microvascular and macrovascular complications[9]. Many studies have shown the presence of oxidative stress in both types of DM through increased production of free radicals and decreased antioxidant defense[6,9]. There is a lot of evidence that based on different characteristics of the oxidative stress; its increased presence in DM can be proven[10].

Free radicals are very unstable due to their high reactivity[9,11]. Due to their nature, they have a short lifetime and are difficult to measure and accurately determine *in vivo*, as well as in biological ma-

TABLE 1
THE DIFFERENCES IN ANTIOXIDANT ENZYMES ACTIVITY AND TOTAL ANTIOXIDANT STATUS (TAS) IN DIABETIC (DM+) AND NON-DIABETIC (DM-) PATIENTS USING THE MANN-WHITNEY TEST

TAS		
Average rank	N	
83.06	109	DM+
130.51	102	DM–
	211	Total
U	z	p
3,058.5	–5.648	0.000
Superoxide dismutase		
Average rank	N	
122.56	109	DM+
88.31	102	DM–
	211	Total
U	z	p
3,754.5	–4.112	0.000
Catalase		
Average rank	N	
95.22	109	DM+
117.52	102	DM–
	211	Total
U	z	p
4,384.0	–2.651	0.008
Glutathione peroxidase		
Average rank	N	
84.29	109	DM+
129.20	102	DM–
	211	Total
U	z	p
3,192.5	–5.341	0.000

TABLE 2
THE DIFFERENCES IN ANTIOXIDANT ENZYMES ACTIVITY AND TOTAL ANTIOXIDANT STATUS (TAS) IN PATIENTS WITH AND WITHOUT PERIPHERAL VASCULAR DISEASE (PVD), ONLY IN GROUPS WITH DIABETES, USING THE MANN-WHITNEY TEST

TAS		
Average rank	N	
54.01	53	PVD+
55.94	56	PVD–
	109	Total
U	z	p
1,431.5	–0.319	0.750
Superoxide dismutase		
Average rank	N	
56.82	53	PVD+
53.28	56	PVD–
	109	Total
U	z	p
1,387.5	–0.590	0.555
Catalase		
Average rank	N	
62.59	53	PVD+
47.81	56	PVD–
	109	Total
U	z	p
1,081.5	–2.440	0.015
Glutathione peroxidase		
Average rank	N	
53.68	53	PVD+
56.25	56	PVD–
	109	Total
U	z	p
1,414.0	–0.424	0.671

terial such as plasma or other body fluids[12]. In clinical states, their existence is determined by their influence on other molecules or antioxidant mechanisms which they cause[12], so it is more reliable to measure consequences of their actions, mostly the decreased level of antioxidant enzymes activity[13].

Antioxidant status was evaluated through the activity of key antioxidant enzymes in patients with PVD with and without DM, and in patients with DM without PVD as well as in the healthy control group. Results of our research indicate a disturbed antioxidant status in diabetic patients as a sign of oxidative stress presence. Literature data also indicates decreased antioxidant reserves in DM, where oxidative stress can play an important role in pathogenesis of diabetic

TABLE 3
THE DIFFERENCES IN ANTIOXIDANT ENZYMES ACTIVITY AND TOTAL ANTIOXIDANT STATUS (TAS) IN PATIENTS WITH AND WITHOUT PERIPHERAL VASCULAR DISEASE (PVD), ONLY IN THE NON-DIABETIC GROUP AND THE HEALTHY GROUP, USING THE MANN-WHITNEY TEST

TAS		
Average rank	N	
51.03	59	PVD+
52.15	43	PVD-
	102	Total
U	z	p
1,240.5	–0.190	0.849
Superoxide dismutase		
Average rank	N	
47.81	59	PVD+
56.56	43	PVD-
	102	Total
U	z	p
1,051.0	–1.495	0.135
Catalase		
Average rank	N	
49.42	59	PVD+
54.36	43	PVD-
	102	Total
U	z	p
1,145.5	–0.834	0.405
Glutathione peroxidase		
Average rank	N	
54.40	59	PVD+
47.52	43	PVD-
	102	Total
U	z	p
1,097.5	–1.159	0.246

vascular complications such as atherosclerotic PVD[14].

In our group of diabetic patients with PVD we have found a decreased activity of antioxidant enzymes, mainly catalase and GLPX, as well as lowered TAS. However, SOD activity has been found to be significantly increased.

Similar results were also found in the group of diabetic patients without PVD. Antioxidant status was equally disturbed in diabetic patients without the regard to PVD presence.

Similar findings were noted by English authors[15] in young diabetic patients that were insulin dependant, where antioxidants glutation and tiol were decreased, while the SOD activity was significantly increased, but without signs of diabetic angiopathy. Researchers from a biochemical research center in Barcelona, Spain, found a significantly increased SOD activity and a decreased GLPX activity in diabetic children and adolescents as compared to the control group[11]. Matching our results for the presence of oxidative stress through changed antioxidant enzymes without the regard to the presence of macrovascular disease, Santini and others also found an increased oxidative stress and decreased antioxidant capacity in their diabetic patients without the regard to the presence of complications[16]. This was also confirmed by a group of other authors[12].

However, another group of authors found an increased oxidative stress even in patients with PVD without DM. Increased malondialdehyde (MDA) as an indicator of lipid peroxidation and decreased tiol is significant in participants with PVD in comparison to the control group, even though inside the group of diabetic participants with microangiopathy, but without PVD, MDA was significantly higher, and tiol was lower as compared to the group with PVD. The group of diabetic patients was significantly younger, and even though they did not develop macrovascular complications, they had a higher level of oxidative stress than the older participants with PVD. Authors believe that oxidative stress in young diabetic patients can contribute to earlier development of macrovascular complications[17]. However, relationship between the glucose concentration and lipid peroxidation is contradictory. Some authors

did not even find a relationship between the glucose concentration and lipid peroxidation in their studies[3], which allows findings of atherosclerosis of major blood vessels without damaging antioxidant status seen in diabetic atherosclerotic disease. In other words, it indicates that lipid peroxidation which leads to endothelium dysfunction is a complex multi -component mechanism, which is supported by our results in the group of non-diabetic patients with PVD and practically regular antioxidant status, represented by main antioxidant enzymes.

In our study of diabetic patients, there was a significant decrease in TAS, as well as in catalase and GLPX activity, which matches most results in literature and is a clear indicator of oxidative stress presence in DM[18]. However, data about some components of antioxidant status, that is, about the activity of antioxidant enzymes is contradictory. Some authors note that SOD activity is decreased when it is exposed to an increased glucose concentration *in vitro*, while an increased activity of a glycated erythrocyte-derived SOD is found in erythrocytes of diabetic patients[19]. However, some studies have shown that their participants under the influence of oxidative stress had an increased lipid peroxidation, and decreased erythrocyte SOD[20,21].

The role of SOD in DM is considered contradictory. Some studies, matching our results, have shown an increased[15,22], while others have shown a decreased SOD activity in diabetic patients[23,24]. Authors in one Scandinavian study estimated oxidative stress in DM type 1 without the regard to the presence or absence of vascular complications and concluded that there was no significant difference in the levels of SOD between the diabetic and control group, while GLPX activity was found to be significantly decreased in diabetic group[25].

Antioxidant enzymes behave differently in different tissues[26]. Applying standardized methods could improve inter-laboratory comparisons[27].

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AKTIVNOST ANTIOKSIDATIVNIH ENZIMA U BOLESNIKA S PERIFERNOM VASKULARNOM BOLESTI, SA I BEZ PRISUSTVA ŠEĆERNE BOLESTI

S A Ž E T A K

U radu je analiziran antioksidativni status u bolesnika s perifernom vaskularnom bolesti (PVB), sa i bez prisustva šećerne bolesti (ŠB). 211 ispitanika podijeljeno je u 4 skupine: ispitanici sa PVB i ŠB (PVB+ŠB+), ispitanici sa PVB bez ŠB (PVB+ŠB-), ispitanici bez PVB sa ŠB (PVB-ŠB+) i ispitanici bez PVB i ŠB (PVB-ŠB-). Dijagnoza PVB postavljena je Doppler sonografskom obradom, koja uključuje mjerenje indeksa tlaka nožnog zgloba (engl. ankle brachial index, ABI), segmentnih tlakova duž noge i CW Doppler sonografije na tipičnim mjestima. Antioksidativni status prikazan je određivanjem serumske aktivnosti ključnih antioksidativnih enzima: superoksid dismutaze (SOD), katalaze, glutation peroksidaze (GLPX) i totalnog antioksidativnog statusa (TAS). U skupini PVB+ŠB+ kao i u skupini PVB-ŠB+ nađene su značajno niže aktivnosti enzima GLPX, katalaze i TAS, dok je aktivnost SOD bila značajno povišena. Nije postojala statistički značajna razlika između PVB+ŠB+ i PVB-ŠB+ skupine. Također, nije bilo značajne razlike između PVB+ŠB- i PVB-ŠB- skupine. Ova studija je pokazala da postoji statistički značajna razlika u aktivnosti antioksidativnih enzima između dijabetičnih i nedijabetičnih ispitanika, bez obzira na prisustvo PVB. Štoviše, prisutna sama, PVB ne mijenja aktivnost ključnih antioksidativnih enzima u usporedbi sa zdravim individuama.