Biološki biljezi enzimske i neenzimske antioksidacijske zaštite u šećernoj bolesti tipa 2 – usporedna analiza

Biomarkers of enzymatic and non-enzymatic antioxidative defense in type 2 diabetes mellitus – comparative analysis

Emina Čolak[1], Vesna Dimitrijević-Srećković[2], Predrag B. Djordjević[2], Sanja Stanković[1], Biljana Glišić[1], Branko Srećković[3], Nada Majkić-Singh[1]

[1]Zavod za medicinsku biokemiju, Klinički centar Srbije, Beograd, Srbija
[1]Institute of Medical Biochemistry, Clinical Center of Serbia, Belgrade,
[2]Zavod za endokrinologiju, dijabetes i bolesti metabolizma, Klinički centar Srbije, Beograd, Srbija
[2]Institute of Endocrinology, Diabetes and Metabolic Disorders, Clinical Center of Serbia, Belgrade,
[3]Zavod za kardiologiju, Klinika za hitnu kardiologiju, Klinički centar Srbije, Beograd, Srbija
[3]Institute of Cardiology, Department of Urgent Cardiology, Clinical Center of Serbia, Belgrade, Serbia

Sažetak

Cilj naše studije bio je odrediti međusobne odnose između „prve", neenzimske, te „druge", enzimske crte antioksidacijske zaštite u bolesnika s šećernom bolesti tipa 2 i očitovanim kardiovaskularnim komplikacijama. Drugi je cilj bio utvrditi odnose između prooksidacijskih (lipidni status) i antioksidacijskih parametara u ispitivanih bolesnika.

Materijali i metode: U našu je studiju parova bilo uključeno ukupno 117 bolesnika s šećernom bolesti tipa (69 sa, te 48 bez kardiovaskularnih komplikacija) i 42 zdrava ispitanika. Određivani su sljedeći antioksidacijski enzimski parametri: eritrociti, Cu, Zn-SOD, glutation-peroksidaza (GPx) i glutation-reduktaza (GR), kao i ukupni antioksidacijski status (engl. *total antioxidant status*, TAS), te bilirubin, mokraćna kiselina, ukupni proteini, albumin i haptoglobin. Enzimski antioksidacijski parametri i TAS analizirani su pomoću komercijalnih testova tvrtke *Randox Ltd*, V. Britanija, koji se temelje na spektrofotometrijskim metodama, dok su ostali neenzimski i lipidni parametri određeni standardnim laboratorijskim metodama.

Rezultati: U odnosu na zdrave ispitanike, bolesnici s šećernom bolesti tipa 2 i kardiovaskularnim komplikacijama imali su značajno niže vrijednosti enzimskih antioksidansa ($P < 0,001$) i više vrijednosti ukupnog bilirubina ($P = 0,050$), mokraćne kiseline ($P < 0,001$) i haptoglobina ($P < 0,001$). Slaba je pozitivna korelacija utvrđena između SOD i GPx ($R = 0,289$, $P = 0,028$) te između SOD i GR ($R = 0,259$, $P = 0,045$), a slaba negativna korelacija zabilježena je između GPx i mokraćne kiseline ($R = -0,35$, $P = 0,009$) te GPx i ukupnog bilirubina ($R = -0,40$, $P = 0,018$). TAS je slabo korelirao s trigliceridima ($R = 0,32$, $P = 0,037$), a GPx i GR su korelirali s HDL-kolesterolom ($R = 0,457$, $P = 0,007$; te $R = 0,466$, $P = 0,001$).

Zaključci: Temeljem dobivenih rezultata može se zaključiti da bolesnici s šećernom bolesti tipa 2 imaju značajno promijenjenu antioksidacijsku zaštitu,

Abstract

The objective of our study was to determine the interrelations between the "first", non-enzymatic, and the "second", enzymatic line of antioxidant defense in patients with type 2 diabetes mellitus and manifested cardiovascular complications. The second aim was to determine the relations between prooxidant (lipid status) and antioxidant parameters in patients under observation.

Methods: In our case-control study, a total of 117 type 2 diabetic patients (69 with and 48 without cardiovascular complications) and 42 healthy subjects were included. Antioxidant enzymatic parameters: erythrocyte, Cu,Zn-SOD, glutathione peroxidase (GPx) and glutathione reductase (GR), as well as total antioxidant status (TAS), bilirubin, uric acid, total proteins, albumin and haptoglobin were determined. The enzymatic antioxidant parameters and TAS were analyzed using commercial tests manufactured by Randox Ltd. UK, based on spectrophotometer methods, while the other non-enzymatic and lipid parameters were determined by standard laboratory methods.

Results: In relation to healthy subjects, type 2 diabetics with cardiovascular complications had significantly lower values of enzymatic antioxidants ($P < 0.001$), and higher values of total bilirubin ($P = 0.050$), uric acid ($P < 0.001$) and haptoglobin ($P < 0.001$). Weak positive correlation was found between SOD and GPx ($R = 0.289$, $P = 0.028$) and between SOD and GR ($R = 0.259$, $P = 0.045$), while weak negative correlation was obtained between GPx and uric acid ($R = -0.35$, $P = 0.009$), GPx and total bilirubine ($R = -0.40$, $P = 0.018$). TAS correlated weakly with triglycerides ($R = 0.32$, $P = 0.037$), while GPx and GR correlated with HDL-cholesterol ($R = 0.457$, $P = 0.007$; and $R = 0.466$, $P = 0.001$).

Conclusions: Based on the obtained results, it may be concluded that type 2 diabetic patients have significantly modified antioxidant defense, with a va-

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uz različiti stupanj neravnoteže između skupa neenzimskih tvari i aktivnosti enzimskih antioksidanasa, koja ovisi o tome jesu li se kardiovaskularne komplikacije pojavile ili ne.

Ključne riječi: oksidacijski stres, antioksidacijska zaštita, šećerna bolest tipa 2, kardiovaskularne komplikacije, usporedna analiza

rying degree of imbalance between the "pool" of non-enzymatic substances and the activity of enzymatic antioxidants, which depends on whether they have or not manifested cardiovascular complications.

Key words: oxidative stress, antioxidant defense, type 2 diabetes mellitus, cardiovascular complications, comparative analysis

Uvod

Razvoj, tijek i komplikacije šećerne bolesti tipa 2 usko su povezani s neravnotežom između prooksidacijskog i antioksidacijskog staničnog oštećenja te promjene redokspotencijala. Oksidacijski stres je kod šećerne bolesti rezultat, kako pojačanog stvaranja slobodnih radikala, tako i smanjene sposobnosti za antioksidacijsku zaštitu (1).

Antioksidacijska zaštita organizma, koja uključuje enzimske i neenzimske tvari, održava ravnotežu stvaranjem slobodnih radikala u granicama homeostaze te sprječava širenje reakcije slobodnih radikala koja može prouzročiti oštećenje tkiva (2).

Superoksidna dismutaza (SOD; E.C. 1.15.1.1) katalizira dismutaciju superoksidnih radikala u vodikov peroksid i molekularni kisik.

Glutation-peroksidaza (GPx; E.C. 1.11.1.9) katalizira redukciju vodikovog peroksida ili organskog hidroperoksida u alkohol u prisutnosti reduciranog glutationa kao davatelja elektrona. Stalno obnavljanje reduciranog glutationa provodi se aktiviranjem glutation-reduktaze (GR; E.C. 1.6.3.2).

Mokraćna kiselina je važan fiziološki antioksidans. Djelovanje slobodnih radikala utječe na oksidaciju mokraćne kiseline u alantoin, dok se vezanjem iona bakra i željeza stvaraju postojani kompleksi čime se smanjuje oksidacijski potencijal tih elemenata kao i širenje reakcija slobodnih radikala. Usto, mokraćna kiselina neutralizira hidroksilni radikal i hipoklornu kiselinu (3).

U uvjetima nižeg parcijalnog tlaka kisika, bilirubin djeluje kao snažan „čistač" peroksilnih radikala (4).

Vezanjem iona željeza i bakra, albumin, feritin, transferin, haptoglobin i ceruloplazmin značajno smanjuju nastanak slobodnih radikala i time štite molekule slobodnih masnih kiselina od peroksidacije. S druge strane, albumin može neutralizirati hipoklornu kiselinu kao i peroksilne radikale (5-7).

Cilj naše studije bio je odrediti međusobne odnose između „prve", neenzimske (mokraćna kiselina, albumin, ukupni proteini, bilirubin, haptoglobin i TAS) i „druge", enzimske (SOD, GPx, GR) crte antioksidacijske zaštite u bolesnika s šećernom bolesti tipa 2 i očitovanim kardiovaskularnim komplikacijama (šećerna bolest, DM + kardiovaskularne komplikacije, KVK). Drugi je cilj bio ustanoviti odnose između prooksidacijskih (lipidni status) i antioksidacijskih parametara u ispitivanih bolesnika usporedbom sa zdra-

Introduction

Development, course and complications of type 2 diabetes mellitus are closely associated with imbalance of pro- and antioxidative cell impairment and change of redox potential. Oxidative stress in diabetes is the result of both increased production of free radicals and reduced capacity of antioxidative defense (1).

Antioxidative defense of organism which involves enzymatic and nonenzymatic substances maintains the balance by generation of free radicals within homeostasis limits and prevents the expansion of free-radical reaction that may cause tissue damage (2).

Superoxide dismutase (SOD; E.C. 1.15.1.1) catalyzes dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen.

Glutathione peroxidase (GPx; E.C. 1.11.1.9) catalyzes reduction of hydrogen peroxide or organic hydroperoxide into alcohol in the presence of reduced glutathione as an electron donor. Constant regeneration of reduced glutathione is carried out by activation of glutathione reductase (GR; E.C. 1.6.3.2).

Uric acid is an important physiological antioxidant. Action of free radicals makes uric acid to be oxidized into allantoin, while iron and copper ion binding produces stable complexes, which reduces the oxidation potential of these elements as well as propagation of free-radical reactions. In addition, uric acid neutralizes hydroxyl radical and hypochloric acid (3).

In conditions of low partial oxygen pressure, bilirubin acts as a potent "scavenger" of peroxyl radicals (4).

Albumin, ferritin, transferrin, haptoglobin and ceruloplasmin, by the binding of iron or copper ions, significantly decrease the production of free radicals, thus protecting the molecules of free fat acids from peroxidation. On the other hand, albumin may neutralize hypochloric acid as well as peroxyl radicals (5-7).

The objective of our study was to determine the interrelations between the "first", non-enzymatic (uric acid, albumin, total proteins, bilirubin, haptoglobin and TAS - total antixidant status) and the "second", enzymatic (SOD, GPx, GR) line of antioxidant defense in patients with type 2 diabetes mellitus (DM) and manifested cardiovascular complications (CVC) (DM + CVC). The second aim was to determine the relations between the pro-oxidant (lipid status) and antioxidant parameters in studied patients, compa-

vim ispitanicima i dijabetičarima bez komplikacija kako bi se analizirao učinak kardiovaskularnih komplikacija na sustav antioksidacijske zaštite.

Materijali i metode

Ispitanici

U našu je studiju parova bilo uključeno 117 bolesnika s šećernom bolesti tipa 2 liječenih u Zavodu za endokrinologiju, dijabetes i metaboličke poremećaje, Klinički centar Srbije, Beograd, (62 muškaraca i 55 žena) te 42 zdrava ispitanika (33 žene i 9 muškaraca) koji su činili kontrolnu skupinu. Šećerna bolest je dijagnosticirana na temelju kliničkih značajki i laboratorijskih nalaza: glikemije nakon gladovanja > 7 mmol/L u dva uzastopna mjerenja te glikemije više od 11,1 mmol/L dva sata nakon oralnog opterećenja glukozom od 75 g. Studija je također uključivala bolesnike s šećernom bolesti tipa 2 koji su u Zavodu bili na oralnoj antidijabetičkoj ili inzulinskoj terapiji više od jedne godine. Kriteriji za hipertenziju bili su sljedeći: sistolički krvni tlak preko 140 mmHg te dijastolički krvni tlak preko 90 mmHg, te podatak da su bolesnici bili uključeni u antihipertenzijsko liječenje dulje od jedne godine.
Zdravi su ispitanici bili odabrani među zaposlenicima Zavoda za medicinsku biokemiju Kliničkog centra Srbije, Beograd, koji su u vrijeme studije bili zdravi, bez ikakvih znakova akutnih ili kroničnih stanja, te koji hranom nisu unosili nikakve dodatne antioksidanse. Ti su ispitanici odabrani između pojedinaca koji su prošli redoviti liječnički pregled i čiji su laboratorijski nalazi ukazali na odsutnost šećerne bolesti, hipertenzije, koronarne bolesti te poremećaja koronarnog stanja.
Svi su ispitanici potpisali informirani pristanak za sudjelovanje u studiji, a dobiveno je odobrenje lokalnog etičkog odbora.

Metode

Uzorci krvi za analizu uzeti su nakon 12–14-satnog gladovanja tijekom noći. Sve laboratorijske pretrage izrađene su neposredno nakon vađenja krvi.
Antioksidacijski parametri SOD, GPx, GR i TAS određeni su komercijalnim testovima tvrtke Randox Ltd., V. Britanija, zasnovanima na spektrofotometrijskim metodama prema Goldsteinu (za SOD) (8), Paglii i Valentineu (za GPx) (9), Milleru (za TAS) (10), te Goldbergu (za GR) (11), dok su za mjerenje ostalih neenzimskih i lipidnih parametara korišteni standardni laboratorijski testovi.
SOD je određivana u krvnom hemolizatu koji je dobiven ispiranjem eritrocita četiri puta s 3 mL NaCl (154 mmol/L) te konačno liziranjem ispranih eritrocita s hladnom deioniziranom vodom i 15-minutnim stajanjem na + 4 °C da bi se dovršio proces hemolize.
Glutation-peroksidaza je mjerena u uzorku pune krvi koji je prije mjerenja razrijeđen 41 puta postupnim dodavaring them to healthy control subjects and complication-free diabetics in order to analyze the impact of cardiovascular complications on the antioxidant defense system.

Materials and methods

Subjects

Our case-control study included 117 type 2 diabetic patients treated at the Institute of Endocrinology, Diabetes and Metabolic Disorders, Clinical Center of Serbia, Belgrade (62 males and 55 females) and 42 healthy subjects (33 women and 9 men), who comprised the control group. The diagnosis of diabetes was made on the basis of patients' clinical features and laboratory findings: fasting glycemia over 7 mmol/L in two subsequent measurings, and/or higher than 11.1 mmol/L two hours after 75 g oral glucose load. The study also included type 2 diabetic patients who were treated in the afore mentioned Institute and were on oral antidiabetic or insulin therapy longer than 1 year. Criteria for hypertension were: systolic blood pressure over 140 mmHg and diastolic blood pressure over 90 mmHg; and/or the information that patients were on some antihypertensive treatment longer than 1 year.
Healthy subjects were recruited from the employees of the Institute of Medical Biochemistry, Clinical Center of Serbia, Belgrade, who were healthy at the time of study, without any signs of acute or chronic conditions and who did not take any additional antioxidants in their food. They were selected among those individuals who went through regular medical check-up and whose laboratory findings showed the absence of diabetes, hypertension, coronary disease or coronary disorder.
All subjects gave their informed consent on participation in the study, and the local ethic committee approved this study.

Samples

The blood samples for analysis were taken after 12–14-hour overnight fast. All laboratory tests were done immediately after sampling.
Antioxidant parameters SOD, GPx, GR and TAS, were determined by commercial tests (Randox Ltd., UK), based on spectrophotometer methods by Goldstein (for SOD) (8), Paglia and Valentine (for GPx) (9), Miller (for TAS) (10) and Goldberg (for GR) (11), while other non-enzymatic and lipid parameters were determined by standard laboratory tests.
SOD was determined in blood hemolysate obtained by washing of erythrocytes 4 times with 3 mL of NaCl (154 mmol/L) and finally by lysing of washed erythrocytes with cold deionized water, followed by a period of 15 minutes at +4 °C to complete hemolysis.
Glutathione peroxidase was determined in the whole blood sample which was, just before determination, dilu-

njem razrjeđivača (dostavljenog uz test) i dvostruko koncentriranog Drabkinovog reagensa.
Glutation-peroksidaza i TAS određivani su u plazmi koja je dobivena centrifugiranjem Li-heparinizirane krvi 10 min brzinom od 3000 okr/min.

Statistička analiza

Rezultati su prikazani kao srednja vrijednost ± SD za kontinuirane varijable s normalnog razdiobom. Statistička analiza razlike između svih skupina provedena je primjenom testa ANOVA. Spearmanov korelacijski test korišten je za definiranje korelacija pojedinačnih parametara između i unutar skupina. Statističke su analize učinjene primjenom statističke programske podrške SPSS v10.0 (SPSS Inc., Chicago, SAD). Svi su statistički testovi bili dvosmjerni. Vrijednosti P ≤ 0,05 smatrane su statistički značajnima.

Rezultati

Vrijednosti ispitivanih parametara te opće informacije o ispitanicima prikazane su u Tablici 1.

ted 41 fold by gradual addition of diluent (supplied in the test kit) and double-concentrated Drabkin's reagent.
Glutathione peroxidase and TAS were determined in plasma that was obtained by centrifugation of Li-heparinized blood 10 min/3000 rpm.

Statistical analysis

Results were presented as mean ± SD for continuous normally distributed variables. Statistical analysis of differences between all groups was performed using ANOVA test. Spearman's correlation test was used to define correlations of individual parameters between and within groups. Statistical analyses were performed using SPSS v10.0 (SPSS Inc. Chicago IL) statistical software. All statistical tests were two-tailed. P values ≤ 0.05 were considered statistically significant.

Results

The values of tested parameters and general information on subjects are presented in Table 1.

Tablica 1. Demografska obilježja i mjereni biokemijski parametri ispitivanih skupina

Table 1. Demographic characteristics and measured biochemical parameters of the groups under observation.

Patient characteristics	DM with complications	DM without complications	Control group	P *
Number of patients	69	48	42	-
Sex (male/female)	37/32	25/23	9/33	-
Age (years)	58 ± 9	58 ± 10	52 ± 11	-
Duration of DM (years)	9 ± 10	7 ± 8	-	-
Smokers (%)	33.3	28.6	40	-
Glucose (mmol/L)	8.6 ± 3.2	9.23 ± 3.4	5.04 ± 0.8	< 0.001
SOD (U/gHb)	806.5 ± 103.6	961 ± 92.9	969.4 ± 104.8	< 0.001
GPx (U/gHb)	23.6 ± 4.6	27.2 ± 5.3	29.1 ± 3.5	< 0.001
GR (U/L)	55.1 ± 9.5	59.3 ± 8.8	62.5 ± 8.0	0.001
TAS (mmol/L)	1.18 ± 0.19	1.27 ± 0.21	1.35 ± 0.23	0.001
Uric acid (µmol/L)	327.3 ± 101	289.5 ± 102.3	251 ± 86.7	0.001
Total bilirubin (µmol/L)	14.8 ± 6.8	13.9 ± 6.4	12.5 ±	0.086
Direct bilirubin (µmol/L)	5.4 ± 2.8	4.3 ± 1.7	4.7 ± 1.3	0.548
Albumin (g/L)	43.5 ± 4.9	44.1 ± 3.9	45.3 ± 4.9	0.091
Total proteins (g/l)	69.6 ± 6.1	71.4 ± 4.5	73.4 ± 4.6	0.003
Haptoglobin (g/L)	1.38 ± 1.28	1.03 ± 0.45	0.74 ± 0.19	0.000
Creatinine (µmol/L)	94.8 ± 26.3	92.2 ± 16.9	87 ± 12.3	0.077
Total cholesterol (mmol/L)	6.07 ± 1.25	6.6 ± 1.6	5.8 ± 0.9	0.006
LDL-cholesterol (mmol/L)	4.0 ± 1.2	4.3 ± 1.4	3.8 ± 0.9	0.050
HDL-cholesterol (mmol/L)	1.15 ± 0.33	1.3 ± 0.32	1.21 ± 0.19	0.990
Triglycerides (mmol/L)	2.58 ± 1.57	2.68 ± 2.83	1.6 ± 0.66	0.006
Apoprotein A1 (g/L)	1.467 ± 0.398	1.67 ± 0.47	1.53 ± 0.28	0.879
Apoprotein A2 (mg/L)	326.7 ± 73.7	346.1 ± 60.6	321 ± 81.2	0.384
Apoprotein B (g/L)	1.299 ± 0.35	1.30 ± 0.27	1.35 ± 0.34	0.454
Apoprotein E (mg/L)	77.5 ± 36.1	73.0 ± 36.9	60.5 ± 56.7	0.001
Apoprotein Lp(a) (g/L)	0.167 ± 0.18	0.29 ± 0.35	0.15 ± 0.08	0.085

* ANOVA test

Od ukupnog broja ispitivanih bolesnika s šećernom bolesti, 48 ispitanika (23 žene i 25 muškaraca) u dobi od 58 ± 10 godina imalo je šećernu bolest tipa 2 bez komplikacija (DM), dok je 69 ispitanika (32 žene i 37 muškaraca) u dobi od 57 ± 9, imalo, prema kriterijima Svjetske zdravstvene organizacije, dijagnosticirane kardiovaskularne komplikacije (DM + KVK) kao što su koronarna bolest (engl. *coronary artery disease*, CAD), hipertenzija (HTA), te anamneza akutnog infarkta miokarda (AIM) tijekom posljednjih 8 godina. Trajanje šećerne bolesti u skupini DM + KVK bilo je 9 ± 10 godina, a komplikacije su bile prisutne tijekom jedne do 32 godine, dok je u skupini DM bez komplikacija šećerna bolest trajala 7 ± 8 godina.

U skupini DM + KVK dokumentirana su 32 slučaja koronarne bolesti s hipertenzijom (46,4%) (CAD + HTA), 17 bolesnika bez hipertenzije (CAD) (24,6%), 7 bolesnika (10,2%) s koronarnom bolešću i anamnezom infarkta miokarda (CAD + AIM), a 13 bolesnika (18,8%) imalo je sve tri navedene komplikacije (CAD + AIM + HTA).

U toj je skupini terapija uključivala propisani način prehrane za 29 bolesnika (42%), 30 bolesnika (43,5%) je dobivalo oralne hipoglikemijske lijekove, dok je 10 bolesnika (14,5%) primalo inzulin u kombinaciji s oralnim hipoglikemicima. U skupini bez komplikacija, 21 se bolesnik (43,75%) morao jedino pridržavati propisanog načina prehrane, dok su ostali (56,25%) dobivali oralne hipoglikemijske lijekove.

Dobivene vrijednosti za SOD, GPx i GR bile su značajno niže u bolesnika s šećernom bolesti tipa 2 i kardiovaskularnim komplikacijama ($P < 0,001$) u usporedbi sa zdravim kontrolnim ispitanicima. Značajna je razlika utvrđena između dviju podskupina bolesnika s šećernom bolesti tipa 2. Enzimski antioksidacijski parametri bili su mnogo niži u podskupini bolesnika s šećernom bolesti tipa 2 i kardiovaskularnim komplikacijama u usporedbi s podskupinom dijabetičara bez komplikacija ($P < 0,001$ za SOD i GPx, te $P = 0,025$ za GR).

Vrijednosti neenzimskih antioksidacijskih tvari bile su različite od vrijednosti enzimskih antioksidacijskih parametara. Koncentracija mokraćne kiseline bila je značajno viša u bolesnika s DM + KVK u odnosu na kontrolne ispitanike ($P < 0,001$), no ne i u odnosu na dijabetičare bez komplikacija (Tablica 1.). Ukupni je bilirubin bio također viši u skupini DM + KVK u odnosu na kontrolnu skupinu ($P = 0,05$), no ne i u odnosu na bolesnike s DM bez komplikacija. Vrijednost direktnog bilirubina nije se značajno razlikovala u odnosu na kontrolnu skupinu, no značajna je razlika nađena među skupinama DM + KVK i DM bez komplikacija. Koncentracija albumina nije se značajno razlikovala među tim skupinama, a koncentracija haptoglobina bila je značajno viša u obje ispitivane skupine u odnosu na kontrolnu skupinu (tj. $P < 0,001$ i $P = 0,001$).

U sve tri skupine bolesnika dobiven je niz značajnih korelacija između enzimskih i neenzimskih parametara. U sku-

Of a total number of studied diabetic patients, 48 subjects, (23 females and 25 males), aged 58 ± 10 years, had type 2 diabetes without complications (DM), while 69 subjects, (32 females and 37 males), aged 57 ± 9 years, were diagnosed with cardiovascular complications (DM + CVC) according to the World Health Organization criteria such as coronary artery disease (CAD), hypertension (HTA) and a personal history of acute myocardial infarction (AMI) in the last 8 years. Duration of diabetes in the DM + CVC group was 9 ± 10 years, and complications lasted from 1 to 32 years, while in the group DM without complications, diabetes lasted 7 ± 8 years.

In the group DM + CVC, 32 cases of coronary disease with hypertension (46.4%) (CAD + HTA), 17 patients without hypertension (CAD) (24.6%), 7 patients (10.2%) with coronary disease and personal history of myocardial infarction (CAD + AMI) were documented, and 13 patients (18.8%) had all three complications (CAD + AMI + HTA).

In the DM + CVC group, the therapy involved dietary regime in 29 patients (42%), and 30 patients (43.5%) were administered oral hypoglycemic drugs, while 10 patients (14.5%) received insulin combined with oral hypoglycemics. In the group without complications, 21 patients (43.75%) had only dietary regime, and the rest (56.25%) were administered oral hypoglycemic drugs.

The obtained SOD, GPx and GR values were significantly lower in type 2 diabetic patients with cardiovascular complications ($P < 0.001$) compared to healthy control subjects. Significant difference was found between two subgroups of type 2 diabetic patients. The enzymatic antioxidant parameters were much lower in the subgroup of type 2 diabetics with cardiovascular complications compared to the subgroup of complication-free diabetic patients ($P < 0.001$ for SOD and GPx and $P = 0.025$ for GR).

The values of enzymatic antioxidant parameters were different from those for non-enzymatic antioxidant substances. Uric acid values were significantly higher in DM + CVC in relation to the controls ($P < 0.001$), but not in relation to DM without complications (Table 1). Total bilirubin was also higher in the group DM + CVC in relation to control group ($P = 0.05$), but not in relation to DM without complications. Direct bilirubin value was not significantly different in relation to control group, but significant difference was found between the groups DM + CVC and DM with no complications. Albumin levels were not significantly different between these groups, while haptoglobin values were significantly higher in both studied groups in relation to the control group ($P < 0.001$ and $P = 0.001$, respectively).

A series of significant correlations between enzymatic and non-enzymatic parameters was obtained in all three groups of subjects. In DM + CVC, weak but significant correlations were obtained between SOD and haptoglobin values ($R = 0.35$, $P = 0.049$); SOD and total proteins ($R =$

pini DM + KVK dobivene su slabe no značajne korelacije između vrijednosti SOD i haptoglobina (R = 0,35, P = 0,049); SOD i ukupnih proteina (R = 0,29, P = 0,049), kao i između TAS i mokraćne kiseline (R = 0,35, P < 0,001), dok su slabe značajne negativne korelacije utvrđene između GPx i mokraćne kiseline (R = –0,35, P = 0,009) te GPx i ukupnog bilirubina (R = –0,40, P = 0,012).

U skupini DM bez komplikacija vrijednosti TAS su umjereno korelirale s bilirubinom (R = 0,54, P < 0,001), a vrijednosti GPx slabo su korelirale s ukupnim proteinima (R = 0,395, P = 0,015).

Koncentracije ukupnog kolesterola bile su slične u obje skupine bolesnika, a značajne su razlike dobivene u usporedbi s kontrolnom skupinom (tj. P = 0,040 i P = 0,001). Koncentracije triglicerida bile su značajno više u obje skupine u odnosu na kontrolne ispitanike, dok su koncentracije HDL- i LDL-kolesterola bile slične u sve tri skupine ispitanika. Koncentracije apoproteina apo A1, apo A2 i apoB bile su također slične u sve tri skupine ispitanika, dok su koncentracije apo E bile značajno niže u kontrolnoj skupini nego u dijabetičara s komplikacijama (P = 0,045).

Vrijednosti TAS slabo su korelirale s trigliceridima (R = 0,32, P = 0,016), dok su aktivnosti GPx i GR pozitivno korelirale s HDL-kolesterolom u obje dijabetičke skupine (R = 0,457, P = 0,007, te R = 0,466, P = 0,001 za DM bez i sa komplikacijama). Slaba, no pozitivna korelacija nađena je između GR i apoproteina A1 (R = 0,289; P = 0,040) u skupini DM + KVK.

U zdravih su ispitanika utvrđene statistički značajne, no slabe korelacije između vrijednosti SOD i ukupnih proteina (R = 0,395, P = 0,001), albumina (R = 0,326, P = 0,039) i mokraćne kiseline (R = 0,378, P = 0,014), a postojala je i slaba obrnuta korelacija između GPx i ukupnih proteina i albumina (R = –0,329, P = 0,037, te R = –0,369, P = 0,019). Glutation-peroksidaza je slabo korelirala s LDL-kolesterolom (R = 0,376, P = 0,028), kao i s apo A2 i apo B (R = 0,477; P = 0,005; te R = 0,381, P = 0,029).

Vrijedno je spomenuti da su vrijednosti TAS i GR bile u slaboj pozitivnoj korelaciji s koncentracijom glukoze u zdravih kontrolnih ispitanika (R = 0,413, P = 0,006, te R = 0,304, P = 0,049), što ukazuje da je porast koncentracije glukoze u serumu u zdravih ispitanika povezan s pojačanom aktivnošću glutation-reduktaze i višim koncentracijama nekih neenzimskih antioksidansa.

U bolesnika s šećernom bolesti bez komplikacija također je zabilježena slaba pozitivna korelacija između GPx i koncentracije glukoze (R = 0.384, P = 0.049), kao i između SOD i glukoze (R = 0.375, P = 0.050), što je također naznačilo povezanost više razine antioksidacijske zaštite i viših koncentracija glukoze u dijabetičara u kojih se nisu razvile komplikacije.

Spearmanov koeficijent korelacije otkrio je slabu negativnu, no značajnu korelaciju između GPx i vrijednosti glukoze (R = –0,382, P = 0,049) u skupini bolesnika s DM, CD i

0.29, P = 0.049) as well as between TAS and uric acid (R = 0.35, P < 0.001), while weak significant negative correlations were found between GPx and uric acid (R = –0.35, P = 0.009) and GPx and total bilirubin (R = –0.40, P = 0.012).

In the group DM without complications, TAS values correlated moderately with bilirubin (R = 0.54, P < 0.001), and GPx values correlated weakly with total proteins (R = 0.395, P = 0.015).

Total cholesterol concentrations were similar in both groups of affected subjects, and significant difference was obtained in comparison to control group (P = 0.040 and P = 0.001, respectively). Triglyceride values were significantly higher in both groups in relation to controls, while HDL and LDL cholesterol levels were similar in all three groups of subjects. Values of apoproteins apo A1, apo A2 and apo B were similar in all three groups of subjects, while apo E concentrations were significantly lower in the control group than in diabetics with complications (P = 0.045).

TAS levels weakly correlated with triglycerides (R = 0.32, P = 0.016) while GPx and GR activities correlated positively with HDL cholesterol in both diabetic groups (R = 0.457, P = 0.007 and R = 0.466, P = 0.001 for DM without and with complications respectively). Weak but positive correlation between GR and apoprotein A1 (R = 0.289; P = 0.040) was found in the DM + CVC group.

In healthy controls, statistically significant but weak correlations were found between SOD values and total proteins (R = 0.395, P = 0.001), albumin (R = 0.326, P = 0.039) and uric acid (R = 0.378, P = 0.014), while there was a weak inverse correlation between GPx and total proteins and albumins (R = –0.329, P = 0.037 and R = –0.369, P = 0.019, respectively). Glutathione peroxidase correlated weakly with LDL-cholesterol (R = 0.376, P = 0.028) as well as with apo A2 and apo B (R = 0.477; P = 0.005; and R = 0.381, P = 0.029, respectively).

It is worth mentioning that TAS and GR values were in weak positive correlation with glucose concentration in healthy controls (R = 0.413, P = 0.006, and R = 0.304, P = 0.049, respectively), which suggested that the increase of serum glucose concentration in healthy controls was associated with higher activity of glutathione reductase and higher concentrations of some non-enzymatic antioxidants.

In diabetics without complications, weak positive correlation between GPx and glucose concentration was also found, as well as between SOD and glucose (R = 0.384 for P = 0.049, and R = 0.375, P = 0.050, respectively), which also indicated the association of higher level of antioxidative defense with higher glucose concentrations in diabetics who had not developed complications.

Spearman's correlation coefficient disclosed weak negative but significant correlation between GPx and glucose values (R = –0.382, P = 0.049) in DM group with CD and HTA, and also in the DM group with CD and AMI (R =

HTA, te također u skupini DM s CD i AIM (R = –0,860, P = 0,041), dok je u skupini DM sa sve tri vrste komplikacija utvrđena značajna umjerena negativna korelacija između koncentracija SOD i glukoze (R = –0,590, P = 0,035).
U zdravih je ispitanika SOD umjereno korelirao s TAS (R = 0,62; P < 0,001), umjerena obrnuta korelacija zapažena je između SOD i GR (R = –0,58; P < 0,001), dok su GR i TAS bili u slaboj negativnoj korelaciji (R = –0,358; P = 0,027).
Antioksidacijski su parametri u dijabetičara s komplikacijama bili u međusobnoj pozitivnoj korelaciji; zapazili smo slabu korelaciju između SOD i GPx (R = 0,289; P = 0,028) te između SOD i GR (R = 0,259; P = 0,045).

Rasprava

Ovo je prva studija u kojoj se uspoređuju „prva" i „druga" crta antioksidacijske zaštite u bolesnika s šećernom bolesti tipa 2, te njihovi odnosi prema prooksidacijskim tvarima u plazmi kao što su parametri lipidnog statusa, kako bi se analizirala povezanost manifestiranih kardiovaskularnih komplikacija i sustava antioksidacijske zaštite.
Na temelju dobivenih rezultata može se zaključiti da je enzimska antioksidacijska zaštita u bolesnika s šećernom bolesti tipa 2 bila značajno smanjena u usporedbi sa zdravim ispitanicima (P < 0,001). Ozbiljnije je smanjenje enzimske antioksidacijske zaštite zapaženo u dijabetičara sa srčanožilnim komplikacijama, nego u onih bez komplikacija (P < 0,001):
Postoji mnogo spojeva u našemu tijelu koji djeluju kao antioksidansi. Mnogi se od njih redovito mjere u svakodnevnoj praksi; primjerice, mokraćna kiselina, bilirubin, albumin, feritin, transferin, ceruloplazmin, haptoglobin itd. U slučajevima neravnoteže između oksidanasa i antioksidanasa organizam nastoji kroz pojačanu sintezu nekih tvari nadoknaditi nižu aktivnost određenih enzima i time tu ravnotežu održava.
Neenzimska antioksidacijska zaštita bila je pojačana u našim ispitanicima, što je potvrđeno korelacijama utvrđenima između nekih neenzimskih antioksidanasa i aktivnosti antioksidacijskih enzima (GPx i mokraćna kiselina, GPx i bilirubin, TAS i GR, TAS i GPx).
TAS je bio u slaboj pozitivnoj korelaciji s koncentracijom mokraćne kiseline i bilirubina, što je sukladno činjenici da ukupan antioksidacijski status, tj. TAS predstavlja zbirno određivanje svih neenzimskih antioksidansa koji mogu neutralizirati vodikov peroksid u plazmi.
Postoji ogromna razlika u opsegu antioksidacijske zaštite između dijabetičara sa i bez kardiovaskularnih komplikacija, što je ukazalo na značajan učinak vrste i trajanja kardiovaskularnih komplikacija na antioksidacijsku zaštitu. U dijabetičara sa srčanožilnim komplikacijama enzimski su antioksidansi bili u međusobnoj pozitivnoj korelaciji, dok su negativno korelirali s neenzimskim antioksidansima (TAS).

–0.860, P = 0.041); while in the DM group with all three types of complications, significant moderate negative correlation was found between SOD and glucose levels (R = –0.590, P = 0.035).
In healthy controls, SOD correlated moderately with TAS (R = 0.62; P < 0.001), and moderate inverse correlation was observed between SOD and GR (R = –0.58; P < 0.001), whereas GR and TAS were in weak negative correlation (R = –0.358; P = 0.027).
In diabetics with complications, the antioxidative parameters were in mutual positive correlation: we observed weak correlation between SOD and GPx (R = 0.289; P = 0.028) and SOD and GR (R = 0.259; P = 0.045).

Discussion

This was the first study to compare the "first" and the "second" line of antioxidant defense in type 2 diabetic patients, and their relations to pro-oxidant plasma substances such as lipid status parameters, in order to analyze the association of manifested cardiovascular complications and antioxidant defense system.
On the basis of the obtained results, it may be concluded that enzymatic antioxidant defense of type 2 diabetic patients was significantly reduced in comparison with healthy controls (P < 0.001). More profound reduction of enzymatic antioxidant defense was observed in diabetics with cardiovascular complications than in diabetics without complications (P < 0.001).
There is a large number of compounds in our body acting as antioxidants. Many of them are determined on regular basis in our everyday practice such as uric acid, bilirubin, albumin, ferritin, transferrin, ceruloplasmin, haptoglobin, etc. In cases of imbalance of oxidants and antioxidants, organism endeavors, by increased synthesis of some substances, to compensate for lower activity of some enzymes and thus maintains the balance.
Non-enzymatic antioxidant defense was increased in our study subjects, which was confirmed by correlations found between certain non-enzymatic antioxidants and the activity of antioxidant enzymes (GPx and uric acid, GPx and bilirubin, TAS and GR, TAS and GPx).
TAS was in weak positive correlation with values of uric acid and bilirubin, which was consistent with the fact that total antioxidant status (TAS) is a sum determination of all non-enzymatic antioxidants that may counteract hydrogen peroxide in plasma.
There was a huge difference in the extent of antioxidant defense between diabetics with and those without cardiovascular complications, which indicated the significant effect of type and duration of cardiovascular complications on antioxidant defense. In diabetics with cardiovascular complications, enzymatic antioxidants were in positive correlation with each other, while they correlated negatively with non-enzymatic antioxidants (TAS).

U zdravih je ispitanika SOD slabo korelirao s nekim neenzimskim antioksidansima kao što su albumin, ukupni proteini i mokraćna kiselina te time ukazao na mogući sinergijski učinak „prve" i „druge" crte antioksidacijske zaštite. U istoj je skupini GPx korelirao obrnuto s ukupnim proteinima i albuminima, no takva korelacija nije zapažena u skupini bolesnika s šećernom bolesti tipa 2 i kardiovaskularnim komplikacijama. U skupini bolesnika s šećernom bolesti tipa 2 i kardiovaskularnim komplikacijama, GPx je negativno korelirao s mokraćnom kiselinom i ukupnim bilirubinom i time naznačio moguću povezanost tih parametara s razvojem kardiovaskularnih komplikacija u dijabetičara.

Pozitivne bi korelacije između GPx i LDL-kolesterola u zdravih ispitanika mogle ukazati na moguću povezanost između viših koncentracija lipida, koje dovode do ubrzane lipidne peroksidacije i potencijalno većeg smanjenja organskih hidroperoksida kao posljedice pojačane aktivnosti GPx. Takva korelacija nije nađena ni u jednoj od dvije skupine dijabetičara, dok su druge značajne korelacije između GPx i HDL-kolesterola (DM + KVK) i GR i HDL-kolesterola (DM) utvrđene.

U kontrolnoj je skupini (fiziološki uvjeti) porast koncentracije glukoze bio povezan s pojačanom aktivnošću GR i razinama TAS, dok je u dijabetičara bez komplikacija povećana koncentracija glukoze bila povezana s većom aktivnošću GPx i SOD. Takva korelacija nije ustanovljena u skupini DM + KVK. Upravo suprotno tome, korelacija između glukoze i SOD bila je negativna u skupini bolesnika s šećernom bolesti tipa 2 i teškim kardiovaskularnim komplikacijama (KB + HTA + AIM). U našim dvjema prethodnim studijama zapazili smo povezanost više koncentracije glukoze s manjom aktivnošću GPx u dijabetičkim podskupinama koje su imale koronarnu bolest s hipertenzijom i akutni infarkt miokarda (12,13). Takav negativan odgovor sustava antioksidacijske zaštite može se povezati s učinkom glikozilacije proteina i utjecajem oksidacijskog stresa na smanjenu katalitičku aktivnost SOD i GPx; oba ta učinka doprinose oštećenoj ukupnoj antioksidacijskoj zaštiti dijabetičara s kardiovaskularnim komplikacijama. Na taj način promijenjene vrijednosti ispitivanih parametara i raznolikost korelacija ukazuju na značajno izmijenjenu antioksidacijsku zaštitu u dijabetičara te naznačuju različiti stupanj neravnoteže ne samo između oksidanasa i antioksidanasa, već također između skupa neenzimskih tvari i aktivnosti enzimskih antioksidansa koja uvelike ovisi o prisutnosti, vrsti i trajanju srčanožilnih komplikacija.

Ostali autori koji su se bavili istom tematikom dobili su slične rezultate. Abou Seif je sa suradnicima utvrdio smanjene aktivnosti SOD, katalaze i ceruloplazmina u bolesnika s šećernom bolesti tipa 2 (14) i povećanim koncentracijama lipidnih parametara kao što su kolesterol, trigliceridi, LDL-kolesterol, te sniženim koncentracijama HDL-kolesterola i povišenim koncentracijama produkata lipidne peroksida-

In healthy subjects SOD correlated weakly with some non-enzymatic antioxidants such as albumin, total proteins and uric acid, suggesting the possible synergistic effect of the "first" and the "second" line of antioxidant defense. In the same group, GPx correlated inversely with total proteins and albumins, but such correlation was not observed in the group of type 2 diabetics with cardiovascular complications. In the group of patients suffering from type 2 diabetes with cardiovascular complications, GPx correlated negatively with uric acid and total bilirubin, thus indicating the possible association of these parameters with the development of cardiovascular complications in diabetics.

Positive correlations between GPx and LDL-cholesterol in healthy subjects could indicate possible association between high lipid concentrations leading to accelerated lipid peroxidation and potentially increased reduction of organic hydroperoxides as a consequence of increased activity of GPx. No such correlation was found in both groups of diabetic patients, while other significant correlations between GPx and HDL-cholesterol (DM + CVC) and GR and HDL-cholesterol (DM) were found.

In the control group (physiological conditions), the increase of glucose concentration was associated with higher GR activity and TAS levels, whereas in diabetics without complications increased glucose level was associated with higher activity of GPx and SOD. Such correlation was not found in the DM + CVC group. On the contrary, correlation between glucose and SOD was negative in the group of type 2 diabetics with severe cardiovascular complications (CAD + HTA + AMI). In two of our previous studies, we observed that higher glucose concentration was associated with lower GPx activity in diabetic subgroups who had coronary disease with hypertension and AMI, respectively (12,13). Such negative response of antioxidative defense system may be related to the effect of protein glycosylation and impact of oxidative stress on reduced catalytic SOD and GPx activity, all of which contributed to impaired total antioxidative defense of diabetics with cardiovascular complications. Thus changed values of tested parameters and diversity of correlations suggest significantly modified antioxidant defense of diabetics, and indicate different degrees of imbalance not only between oxidants and antioxidants but also between the "pool" of non-enzymatic substances and the activity of enzymatic antioxidants that largely depend upon the presence, type and duration of cardiovascular complications.

Other authors who studied this subject have obtained similar results. Reduced activities of SOD, catalase and ceruloplasmin were also obtained by Abou Seif and his co-workers in type 2 diabetics (14), with increased concentrations of lipid parameters such as cholesterol, triglycerides, LDL-cholesterol, lowered values of HDL-cholesterol and increased concentrations of lipid peroxidation and

cije i glikozilacije. Evereklioglu (15) je pokazao da dijabetičari s makularnom degeneracijom imaju manju aktivnost SOD, GPx i više koncentracije MDA i NO u odnosu na zdrave ispitanike. Bolesnici s duljom makulopatijom imali su veće smanjenje SOD i GPx u odnosu na bolesnike u ranoj fazi makulopatije. Martin-Gallan (16) je dokazao da mladi dijabetičari s nedavno otkrivenom mikroangiopatijom imaju značajno snižene vrijednosti GPx, glutationa i β-karotena u odnosu na kontrolne ispitanike, ali ne i u odnosu na dijabetičare bez mikroangiopatije; osim toga, vrijednosti SOD bile su značajno više u obje skupine ispitanika bez obzira na prisutnost ili odsutnost mikroangiopatije. Valabhji i sur. zapazili su niže vrijednosti TAS u bolesnika s šećernom bolesti tipa 1 u odnosu na kontrolne ispitanike; TAS je bio u negativnoj korelaciji s vrijednostima HbA1c (P = 0,0026), trajanjem šećerne bolesti te starenjem, osobito u muškaraca (17). Stariji bolesnici s šećernom bolesti tipa 1 koji su pokazali viši stupanj arterijske kalcifikacije imali su više vrijednosti krvnog tlaka, dulje trajanje dijabetičkog poremećaja, više koncentracije serumskog kolesterola i kreatinina, te niže vrijednosti TAS u usporedbi s bolesnicima sa slabijom kalcifikacijom arterija. Ruiz (18) je sa svojim suradnicima otkrio da dijabetičari ovisni o inzulinu imaju značajno manju aktivnost GPx te da to smanjenje izravno ovisi o stupnju metaboličke kontrole. Isti je autor potvrdio da postoji značajna korelacija neenzimskih antioksidansa i koncentracija ukupnog kolesterola, lipidnog hidroperoksida, triglicerida i koncentracija HbA1c. Nojiri i sur (19) su dokazali da su vrijednosti TAS i koncentracije retinala, albumina, ukupnih proteina i HDL-kolesterola bile značajno niže u bolesnika s koronarnom bolešću u usporedbi s kontrolnim ispitanicima. Vrijednosti TAS pozitivno su korelirale s mokraćnom kiselinom i negativno s brojem zahvaćenih žila. Njihova je studija dokazala povezanost antioksidacijskih parametara i napredovanja ateroskleroze, no nije uspjela potvrditi antioksidanse kao neovisan čimbenik rizika za koronarni događaj.

U našoj smo studiji prikazali povezanost nekih enzimskih antioksidansa s ukupnom neenzimskom antioksidacijskom aktivnošću plazme i pojedinačnih neenzimskih antioksidacijskih tvari. Odabrali smo tvari s antioksidacijskom aktivnošću koje se najčešće određuju u postojećoj laboratorijskoj praksi. Osim tih tvari s antioksidacijskim učinkom, druge tvari (tj. glutation, α-tokoferol, zeaksantin, likopen, ubikvinol i dr.) također predstavljaju ukupnu antioksidacijsku sposobnost plazme; one nisu bile uključene u studiju, što predstavlja njeno moguće ograničenje.

Zaključak

Ukratko, povišeni oksidacijski stres i oksidacijsko oštećenje tkiva predstavljaju uobičajene posljedice kroničnih bolesti kao što su ateroskleroza i šećerna bolest. Postoji veliki broj dokaza da su biokemijski putovi, na koje nepo-

glycosylation products. Evereklioglu (15) showed that diabetics with macular degeneration had lower activity of SOD, GPx and higher concentrations of MDA and NO in relation to healthy subjects. Patients with longer maculopathy had larger reduction of SOD and GPx in relation to those in the early phase of maculopathy. Martin-Gallan (16) proved that young diabetics with newly detected microangiopathy had significantly reduced values of GPx, reduced glutathione and β-carotene in relation to the controls, but not in relation to diabetics without microangiopathy; in addition, SOD values were significantly higher in both groups of subjects regardless of the presence or absence of microangiopathy. Valabhji and his co-workers observed lower TAS values in type 1 DM in relation to the controls, and those values were in negative correlation with HbA1c values (P = 0.0026), duration of diabetes and aging, particularly in men (17). Older patients with type 1 DM who manifested higher degree of arterial calcification, had higher values of blood pressure, longer duration of diabetic disorder, higher concentrations of serum cholesterol and creatinine and lower values of TAS in comparison with patients with minor arterial calcification. Ruiz (18) and his colleagues found that insulin-dependent diabetics had significantly lower GPx activity and that its reduction directly depended upon the degree of metabolic control. The same author verified that there was a significant correlation of non-enzymatic antioxidants and total cholesterol levels, lipid hydroperoxide, triglyceride and HBA1c concentrations. Nojiri et al (19) demonstrated that TAS values and the concentrations of retinal, albumin, total proteins and HDL-cholesterol were significantly lower in patients with coronary artery disease compared to control subjects. TAS values correlated positively with uric acid and negatively with the number of diseased vessels. Their study demonstrated the association of antioxidant parameters with atherosclerosis progression, but failed to confirm antioxidants as an independent risk factor of CAD event.

In our study, we illustrated the association of some enzymatic antioxidants both with total non-enzymatic antioxidant activity of plasma and individual non-enzymatic antioxidant substances. We chose those substances with antioxidant activity which are most usually determined in current laboratory practice. Besides these substances with antioxidant effect, others (i.e. glutathione, α-tocopherol, zeaxantine, lycopene, ubiquinol and others) also represent the total antioxidant capacity of plasma; they were not included, which is a possible limitation to this study.

Conclusion

In summary, elevated oxidative stress and oxidative tissue damage are common end points of chronic diseases such as atherosclerosis and diabetes. There is considerable evidence that many biochemical pathways adversely affec-

voljno utječu hiperglikemija i ostale tvari koje se u povišenim koncentracijama nalaze u dijabetičara, povezani s nastankom reaktivnih kisikovih radikala te da konačno dovode do pojačanog oksidacijskog stresa u raznim tkivima. Dijabetičari s krvožilnim komplikacijama mogu imati manjkav stanični antioksidacijski odgovor na oksidacijski stres nastao zbog hiperglikemije. Time je ujedno potaknuta zamisao da bi antioksidacijska terapija mogla biti od značajnog interesa za te bolesnike. Stoga su potrebna daljnja istraživanja terapijskih strategija za sprječavanje ili odgađanje razvoja dijabetičkih krvožilnih komplikacija.

ted by hyperglycemia and other substances that are found at elevated levels in diabetic patients are associated with the generation of reactive oxygen species, ultimately leading to increased oxidative stress in a variety of tissues. Diabetic patients with vascular complications may have a defective cellular antioxidant response against the oxidative stress generated by hyperglycemia. Such view encourages the idea that antioxidant therapy of these patients may be of great interest. Thus, further investigations of therapeutic strategies to prevent or delay the progression of diabetic vascular complications are needed.

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Adresa za dopisivanje:
Dr. Emina Colak, MD
Institute of Medical Biochemistry
Clinical Center of Serbia
Visegradska 26
11000 Belgrade
Serbia
e-pošta: eminacolak@sbb.co.yu
tel: +381 11 3615 631

Corresponding author:
Dr. Emina Colak, MD
Institute of Medical Biochemistry
Clinical Center of Serbia
Visegradska 26
11000 Belgrade
Serbia
e-mail: eminacolak@sbb.co.yu
phone: +381 11 361 56 31

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