BIOMEDICINSKA ISTRAŽIVANJA
2018;9(1):27–36

UDK: 616.61:616.379-008.64
DOI: 10.7251/BII1801027M

Original article

Oxidative stress and other risk factors associated with diabetic nephropathy in type 2 diabetes mellitus

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Primljen – Received: 25/04/2018
Prihvaćen – Accepted: 21/05/2018

Summary

Introduction. The aim of the study was to examine whether biomarkers of oxidative stress and antioxidant enzyme activities are among other risk factors for diabetic nephropathy (DN).

Methods. The study involved 70 patients with type 2 diabetes (37 males, aged 41 to 81 years) allocated to two groups: one of 32 patients with DN and the other of 38 patients without DN. In the study of oxidative stress 15 healthy persons were included. All examined patients were interviewed and underwent objective examination. Their serum and urine samples were analyzed in order to estimate the quality of glycoregulation and kidney function. Protein thiol groups (P-SH), antioxidant enzyme activities [superoxide dismutase (SOD) and glutathione peroxidase (GPX)] were determined in plasma spectrophotometrically and malondialdehyde-adducts (MDA) by enzyme immunoassay.

Results. No significant differences were found between the two groups for demographic characteristics, duration and treatment of diabetes, blood pressure, fasting glucose level and HbA1c. Patients with DN had a higher body mass index, lower estimated glomerular filtration rate (eGFR) and higher albuminuria and proteinuria. Plasma activity of GPX and SOD as well as levels of MDA adducts and P-SH groups were similar in patients with and without DN, but GPX and SOD plasma activities were significantly lower and plasma level of MDA significantly higher in all patients than in healthy controls. Patient gender, age, BMI, HbA1c and plasma level of P-SH and MDA were selected as significant predictors of DN. Patient age, duration of diabetes, serum phosphorus, uric acid levels and plasma SOD activity were negatively associated with eGFR. Patient age, serum levels of protein and albumin and plasma GPX activity were negatively, while systolic BP, serum levels of uric acid and cholesterol were positively associated with proteinuria.

Conclusion. Biomarkers of oxidative protein and lipid damage were selected as risk factors for DN, besides several other well known risk factors.

Keywords: diabetic nephropathy, antioxidant enzymes, oxidative damage biomarkers

Introduction

The incidence of diabetes mellitus is rising all over the world and it is assumed that the number of patients with diabetes will increase from 382 million in 2013 to 592 million in 2035 [1]. The majority of these patients have type 2 diabetes and only about 6% of adults with diabetes have the type 1 diabetes [2]. Both types of diabetes are strongly associated with microvascular and macrovascular complications that significantly impair quality of life.

As one of the microvascular complications of diabetes, diabetic nephropathy (DN) became a serious health and economic problem in developed countries in the late nineties. Due to the steady increase in the number of patients with DN, especially among those with type 2 diabetes, this has become the most common cause of end-stage renal disease (ESRD) [3, 4]. Moreover, at the beginning of this century, increased incidence of ESRD caused by DN was observed all over the world. By 2015 DN had become the leading cause of ESRD in incident (30.4%) patients on renal replacement therapy in Bosnia and Herzegovina [5]. The increasing incidence of DN can only be addressed by persistent implementation of preventive measures. These should be directed towards the risk factors for development and progression of the disease. Although chronic hyperglycemia and glomerular hypertension are considered as the main initiators of DN, many other factors have been shown to contribute to its development [6, 7]. Despite numerous studies on the pathogenesis of and factors affecting DN, there are still not enough effective measures to prevent the occurrence and progression of the disease. Therefore, new risk factors and measures that could improve the outcome of DN by influencing these factors are still sought. Among the many investigated risk factors for DN, oxidative stress seems to mediate macrovascular and microvascular complications of diabetes. Chronic hyperglycemia in diabetic patients causes increased production of oxygen free radicals and depletion of antioxidants. This may lead to increased oxidant-derived tissue injury [8-10].

The aim of this study was to examine risk factors associated with DN in patients with type 2 diabetes, especially taking into account the oxidative status of these patients evaluated through plasma activities of antioxidant enzymes and biomarkers of oxidative damage.

Methods

Patients. The cross-sectional study involved 70 patients with type 2 diabetes (37 males, aged 41 to 81 years) regularly controlled in the Center of Internal Medicine, University Hospital, Foča. Patients were allocated to two groups. Group 1 consisted of 32 patients with DN and group 2 comprised 38 patients without DN. Diagnosis of DN was based on the presence of albuminuria (urine albumin to creatinine ratio > 3.4 mg/mmol) and/or proteinuria (urine protein to creatinine ratio > 20 mg/mmol) with or without decreased estimated glomerular filtration rate (eGFR < 60 ml/min/1.73m^2). Patients with acute infections, existing urinary tract infection, other kidney diseases, other endocrine diseases and congestive heart failure were not included in the study. None of the examined participants used antioxidant supplements.

In the study of antioxidant enzymes and biomarkers of oxidative damage a third group of 15 healthy persons was included, all of whom had a negative history of diabetes, kidney disease and hypertension, while clinical, laboratory and sonographic examinations revealed no pathological finding.

The study was approved by the Ethical Committee of the Faculty of Medicine, Foča. Informed consent was obtained from all examined persons.

Data on demographic characteristics of the patients, duration and treatment of diabetes, together with family history, were obtained by interview and analysis of medical records. All examined patients underwent an objective examination including blood pressure measurement and calculation of body mass index (BMI).

Laboratory analyses. Blood and urine samples were collected and the following analy-

ses were performed using routine biochemical methods: peripheral blood cell counts, serum levels of fasting and 2-h postprandial glucose, HbA1c, cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, albumin, protein, urea, creatinine, uric acid, calcium and phosphorus, as well as urine creatinine level.

eGFR was estimated using the abbreviated MDRD study equation for non standardized creatinine [11]:

$eGFR\ (mL/min/1.73\ m^2) = 186 \times (Scr)^{-1.154} \times (Age)^{-0.203} \times (0.742\ if\ female)$

Urine protein was measured by a colorimetric method with pyrogallol red and expressed as mg protein/mmol creatinine and urine albumin by a photometric color method with bromcresol green on an Olympus AU 400 analyzer (Olympus Co. Ltd., Tokyo, Japan).

Superoxide dismutase (SOD) activity in plasma was measured by the method of Misra and Fridovich [12] based on the ability of SOD to inhibit auto-oxidation of epinephrine at an alkaline pH (pH 10.2). Plasma glutathione peroxidase (GPX) activity was assayed according to Gunzler et al. [13] with organic hydroperoxide as the substrate. Plasma protein thiol groups (P-SH) were determined using the method of Jocelyn [14] and expressed as mmol/g of protein. Plasma MDA-adducts were determined by ELISA using the Oxiselect MDA adduct ELISA kit (Cell Biolabs, San Diego, CA, USA) according to the manufacturer's protocol. MDA was expressed as pmol/mg of protein.

Statistical analysis. Descriptive statistics are reported as frequencies for categorical variables and as means with standard deviation for continuous variables. Comparison of the variables among the groups was made with Student's t test, the Chi^2-test, Fisher's exact test, and one-way analysis of variance (ANOVA) accompanied by Bonferroni multiple comparison tests depending on the variables compared. Univariate/multivariate logistic regression analysis was used to find out variables associated with "belonging to the group" as the dependent variable: belonging to the DN group was coded as 1 and belonging to the group without DN was coded as 0. Univariate/multivariate linear regression was used to detect variables associated with eGFR and urine protein excretion. Demographic, clinical and laboratory variables determined in the study were used as independent variables in both these analyses.

Results

Table 1 shows no significant differences between patients with DN and those without DN for demographic characteristics, duration and treatment of diabetes, or systolic and diastolic blood pressure. The only significant difference was found for BMI, which was higher in patients with DN than in those without DN.

Laboratory analyses revealed that the examined groups differed significantly only for kid-

Table 1. Main characteristics of the patients

	Patients		p*
	with diabetic nephropathy	without diabetic nephropathy	
Number	32	38	
Gender, males	20 (62.5%)	17 (44.7%)	0.138
Age, years	63.5 ± 11.8	60.3 ± 11.5	0.255
Duration of DM, years	10.5 ± 7.2	8.7 ± 7.3	0.305
Therapy:			
medicamentous	14 (43.8%)	17 (44.7%)	0.966
insulin	7 (21.9%)	9 (23.7%)	
combined	11 (34.4%)	12 (31.6%)	
BMI, kg/m^2	29.4 ± 4.3	26.6 ± 3.8	**0.004**
Systolic BP, mmHg	147.9 ± 27.5	139.6 ± 19	0.200
Diastolic BP, mmHg	84.7 ± 10.5	85.3 ± 10.8	0.822

*Statistical significance of differences was calculated by Chi^2 test and Student's t-test, as appropriate. DM – diabetes mellitus, BMI – body mass index, BP - blood pressure

Tabela 2. Results of laboratory analyses

	Patients		
	with diabetic nephropathy n = 32	without diabetic nephropathy n = 38	p
Hemoglobin, g/L	128.5 ± 20.4	135.5 ± 16.3	0.116
Leukocytes x 109/L	8.1 ± 2.7	6.7 ±1.9	**0.01**
Fasting glucose, mmol/L	9.6 ± 2.9	9.2 ± 3.2	0.571
2-h postprandial glucose, mmol/L	11.5 ± 3.2	11.8 ± 3.6	0.718
HbA1c %	8.1 ± 1.5	8.3 ± 1.7	0.733
Cholesterol, mmol/L	5.9 ±1.6	5.7 ± 1.1	0.541
Triglyceride, mmol/L	2.4 ± 0.9	2.3 ± 1.2	0.762
HDL-cholesterol, mmol/L	1.0 ± 0.3	1.0 ± 0.2	0.488
LDL- cholesterol, mmol/L	4.1 ± 1.5	4.0 ±1.0	0.580
S-protein, g/L	63.3 ± 8.0	67.4 ± 7.0	0.028
S-albumin, g/L	36.5 ± 6.3	38.0 ± 4.7	0.274
S-creatinine, µmol/L	112.5 ± 83.8	81.3 ± 20.0	**0.026**
eGFR, ml/min/1.73m^2	63.6 ± 21.5	79.3 ± 20.7	**0.003**
S-uric acid, mmol/L	304 ±107	273 ± 84	0.186
S-calcium, mmol/L	2.1 ± 0.1	2.2 ± 0.2	0.065
S-phosphorus, mmol/L	0.9 ± 0.3	0.8 ± 0.2	**0.003**
U-albumin, mg/g	43.7 ± 10.8	3.0 ± 2.1	**<0.001**
U-protein, mg/g	166.3 ± 183.2	19.4 ± 17.9	**<0.001**

ney function. Patients with DN had significantly higher serum creatinine and phosphorus levels, lower eGFR, and greater urinary excretion of albumin and protein than the patients without DN (Table 2). Analysis of the distribution of patients according to eGFR showed that 14/32 of those with DN had eGFR below 60 ml/min/1.73m^2 but only 4/38 participants without DN (p = 0.0081) Comparison of plasma antioxidant enzyme activities and oxidative damage biomarkers among the three groups examined showed similar values for patients with and without DN (Table 3). However, both patient groups had significantly lower plasma activities of GPX and SOD, but significantly higher levels of MDA adducts than the healthy controls. Although the plasma level of P-SH groups in our patients tended to be lower than in healthy persons, the differences did not reach statistical significance.

Although patients with and without DN had similar values for plasma SOD activity and P-SH levels, the distribution of these parameters differed between the groups with and without DN (Figure 1). While almost all patients without DN had SOD activity below 30 U x 103/L, SOD activity was above this

Table 3. Antioxidant enzymes activities and oxidative damage biomarkers in plasma of patients with/without diabetic nephropathy (DN) and healthy controls

		GPX activity, U/L	SOD activity, Ux10^3/L	MDA adducts, pmol/mg protein	P-SH groups, µmol/g protein
1. Patients with DN (n=32)		304.3 ± 76.0	30.1 ± 13.3	81.2 ± 10.6	7.42 ± 1.24
2. Patients without DN (n=38)		302.5 ± 69.0	25.3 ± 9.7	81.0 ± 11.5	7.72 ± 1.86
3. Healthy controls (n=15)		410.6 ± 131.7	47.1 ± 7.57	65.3 ± 7.38	8.77 ± 2.15
p (ANOVA)		<0.0001	<0.0001	<0.0001	0.020
p (Bonferroni)	1 vs. 2	1.000	0.183	1.000	0.401
	1 vs. 3	<0.0001	<0.0001	<0.0001	0.170
	2 vs. 3	<0.0001	<0.0001	<0.0001	0.209

GPX – glutathione peroxidase, SOD – superoxide dismutase,
P-SH – protein thiol groups, MDA – malondialdehyde-adducts

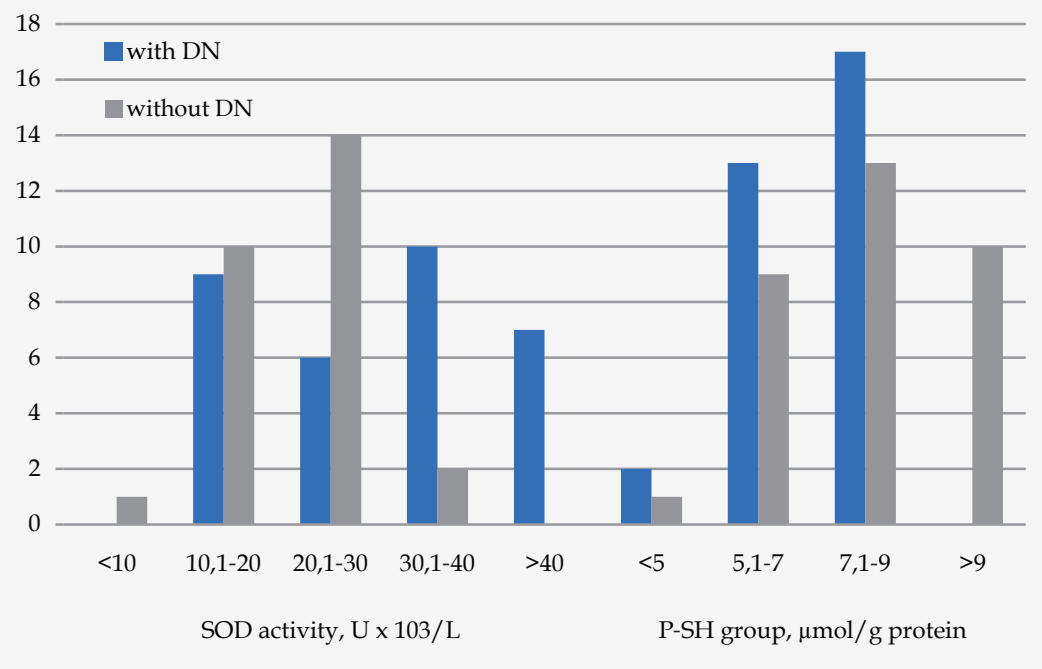

Figure 1. Distribution of patients with and without diabetic nephropathy (DN) according to plasma superoxide dismutase (SOD) activity and level of protein thiol groups (P-SH)

value in more than half of the patients with DN ($p = 0.002$). Moreover, plasma P-SH levels were below 9 µmol/g protein in all patients with DN, but 9/38 patients without DN had P-SH levels above 9 µmol/g protein. This difference in distrubution between the two groups of patients was near statistical significance.

In order to detect risk factors for DN, univariate/multivariate logistic regression analysis was used. The results presented in Table 4 show that males, older patients, those with higher BMI and HbA1c had a greater risk for DN, as well as those with lower plasma P-SH levels and MDA adducts.

Univariate/multivariate linear regression was used to detect variables associated with eGFR and urine protein excretion. Combining variables of both patient groups showed that patient age, duration of diabetes, serum phosphorus and uric acid levels and plasma SOD activity were negatively associated with eGFR.

Patient age, serum levels of protein and

Table 4. Variables associated with belonging to the group of patients with diabetic nephropathy

	B	p	OR	95% CI
Gender, males	3.03	0.003	20.75	2.75-156.45
Age, years	0.09	0.045	1.09	1.00-1.18
BMI, kg/m^2	0.41	0.002	1.51	1.17-1.95
HbA1c, %	0.65	0.063	1.91	0.97-1.09
P-SH, µmol/g protein	–0.86	0.006	0.42	0.23-0.79
MDA, pmol/mg protein	–0.78	0.021	0.92	0.85-0.99

P-SH – protein thiol groups, MDA – malondialdehyde-adducts
BHI – Body mass index

Table 5. Variables associated with eGFR and urinary protein excretion

	eGFR, ml/min/1.73m^2			U-protein, mg/mmol		
	B	β	p	B	β	p
Age, years	-0.47	-0.25	0.038	-3.65	-0.025	0.042
Duration of DM, years	-0.83	-0.27	0.024			
Systolic BP, mmHg				2.93	0.40	0.001
S-phosphorus, mmol/L	-52.63	-0.54	<0.0001			
S-uric acid, mmol/L	-0.07	-0.03	0.03	0.48	0.26	0.030
S-protein, g/L				-9.32	-0.41	0.001
S-albumin, g/L				-10.01	-0.31	0.009
S-cholesterol, mmol/L				37.12	0.28	0.018
GPX activity, U/l				-0.23	-0.12	0.015
SOD activity, Ux103/l	-0.52	-0.28	0.021			

DM – diabetes mellitus, BP – blood pressure, GPX – glutathione peroxidase, SOD – superoxide dismutase

albumin and GPX plasma activity were negatively and systolic BP, serum uric acid and cholesterol concentrations positively associated with urine protein excretion.

Discussion

In our cross sectional study two groups of patients with type 2 diabetes were examined, one with DN and the other without this microvascular complication. The gender, age, duration and treatment of diabetes, blood pressure, fasting glucose and HbA1c levels were similar in both groups. However, values for BMI, albuminuria and proteinuria were higher, while eGFR was lower in the group with DN when compared with the group without DN. While activities of the antioxidant enzymes, GPX and SOD, were similar in both groups with diabetes, they were markedly lower than in the group of healthy persons. In addition, plasma levels of MDA adducts were significantly higher in each group of patients with diabetes than in the healthy controls, whereas plasma P-SH levels tended to be lower. Multivariate logistic regression analysis selected patient gender, age, BMI, HbA1c and plasma levels of P-SH and MDA adducts as significant independent predictors of DN. Patient age, duration of diabetes, serum phosphorus and uric acid levels and plasma SOD activity were negatively associated with eGFR, but patient age, serum levels of protein and albumin and GPX plasma activity were negatively associated with proteinuria, while systolic BP, and serum uric acid and cholesterol were positively associated with proteinuria.

Numerous studies have been devoted to discovering risk factors for DN with special attention to potentially modifiable risk factors as targets of preventive measures. In this context sustained hyperglycemia and hypertension were shown to be the main modifiable risk factors of DN [15, 16]. In the present study no significant differences were found in fasting serum glucose level and HbA1c between patients with and without DN, but mean values of these parameters were above those recommended by modern guidelines [17-19]. Analysis of the distribution of patients according to fasting serum glucose level and HbA1c showed differences between the two examined groups. Thus, 28/32 patients with DN had fasting glucose level above 8 mmol/L but only 17/38 patients without DN (p = 0.0034), while HbA1c below 7% was recorded for 19/32 patients with DN but for 29/38 patients without DN (p = 0.196; data not presented). This indicates that patients with DN had poorer glycoregulation than those in whom DN was not developed. Moreover, multivariate logistic regression selected HbA1c as a significant independent predictor of DN. This confirms numerous randomized control studies showing that intensive glucose control reduced the

rate of development of micro and macroalbuminuria [20-22].

The link between obesity and type 2 diabetes led to creation of the term „diabesity" in order to underline that obesity is the main risk factor for type 2 diabetes and often precedes actual diabetes. However, the results concerning association of obesity and DN are controversial [23-25]. Both groups of patients presented here had mean BMI above the normal limit, but the mean value was significantly higher for those with DN than for those without DN. In addition, 14/32 patients with DN were obese (BMI > 30 kg/m2), but that was the case for only 5/38 patients without DN ($p = 0.0094$). This indicated an association between obesity and DN, which was confirmed by logistic regression analysis that selected BMI as a significant predictor of DN.

Hypertension is common in diabetic patients, especially in those with type 2 diabetes. More than 70% of patients from both groups examined here had hypertension and similar percentages of diabetic patients with hypertension were reported earlier [26, 27]. Systolic blood pressure was higher in our patients with DN than in those without DN and those with DN had had hypertension for a slightly longer time than those without DN (10.4 ± 7.4 vs. 9.8 ± 5.9 years) but the difference was not significant. It may be noted that the duration of hypertension was similar to the reported duration of type 2 diabetes. In patients with this type of diabetes most often it cannot be established when the disease started. It commences asymptomatically and is most often detected when microvascular complications already exist. That could partially explain the similar duration of diabetes in our groups with and without DN. Although the durations of diabetes and hypertension were similar in both our patient groups, duration of diabetes was selected as a significant predictor of eGFR and systolic blood pressure as a significant predictor of proteinuria. Altogether these results indicate the importance of close control of glycemia and blood pressure in patients with diabetes according to guideline recommendations.

While our results showed similar HbA1c values and similar duration of diabetes in patients with and without DN, both these variables appeared as predictors of DN. Therefore, it could be assumed that there are still some disorders associated with hyperglycemia contributing to DN development. Among the many disorders associated with hyperglycemia is the generation of excessive intracellular reactive oxygen species (ROS) [28, 29]. Some cells are particularly susceptible to hyperglycemia due to their inability to decrease glucose transport and prevent large increases in intracellular glucose concentration. Among such cell populations are retinal capillary endothelial cells and some kidney cells, including glomerular epithelial cells, mesangial cells and proximal tubular epithelial cells [29]. In addition, increased ROS production, decreases in the activity of antioxidant enzymes as well as the activity of some antioxidants have been reported in DN [30-32], although studies on the activity of antioxidant enzymes in DN are scarce and show controversial results. Thus, Ozdemir et al.[33] found no significant difference in plasma activity of GPX between patients with microalbuminuria and those without microalbuminuria. On the contrary, Sedighi et al. [34] observed significantly lower plasma GPX activity in diabetic patients with microalbuminuria and an even greater decline in those with macroalbuminuria. Similarly, Kumawat et al. [35] reported significantly reduced plasma activity of GPX and SOD in type 2 diabetes cases with and without DN when compared to controls and also between patient groups with and without DN. These disparate results can be partially explained by the use of various methods to examine antioxidant enzymes, as well as differences in the characteristics of the patients. Our results showed no differences in plasma activity of GPX and SOD between patients with and without DN but they were significantly lower than in healthy controls. However, almost all patients without DN had SOD activity less than 30 U/L, while in the group of patients with DN over 50% of patients had SOD activity above this limit. It can be assumed that the difference in mean SOD activity between our groups of patients with or without DN was not statistically significant due to the

relatively small number of subjects involved. However, numerous studies have shown that GPX activity declines with decreasing kidney function in patients with chronic kidney disease, while for SOD such correlations are not proven [36, 37]. Mimić-Oka et al. [36] even noted that SOD activity in plasma increased with progression of kidney failure. As 41 % of our patients with DN had eGFR below 60 ml/min/1.73m^2, the influence of decreased kidney function on plasma SOD activity in patients with DN should be taken into account.

In this study mean plasma level of MDA adducts was significantly higher in patients with diabetes than in healthy controls but no difference was found between patients with and without DN. Some authors obtained similar results [38], but others found significantly higher plasma MDA levels in patients with DN than in those without DN [33, 35]. Examination of oxidative protein damage gave slightly different results from those for oxidative lipid damage. Both groups of our diabetics had lower plasma levels of P-SH groups than healthy persons but the differences did not attain statistical significance. Similar results were obtained earlier, including for patients with type 1 diabetes [39]. Nevertheless, all patients with DN had plasma P-SH levels below 9 μmol/g protein, which was not the case for our patients without DN. In addition, plasma level of P-SH groups appeared as a significant predictor of DN.

Conclusion

Two groups of patients with type 2 diabetes, one with and the other without DN, did not differ in demographic characteristics, duration and treatment of diabetes, blood pressure, fasting glucose level and HbA1c, while the group with DN had significantly higher mean BMI than that without DN. Patients with DN had also higher urine excretion of albumin and protein and lower eGFR when compared to those without DN. The activity of antioxidant enzymes, GPX and SOD, as well as plasma levels of MDA and P-SH groups were similar in patients with DN and those without DN. However, plasma level of MDA and P-SH groups appeared as significant predictors of DN, while plasma activity of GPX was significantly associated with eGFR and SOD with proteinuria. Male gender, age, BMI and HbA1c were also selected as significant predictors of DN. Age, duration of diabetes, serum phosphorus and uric acid levels and plasma SOD activity were significantly associated with eGFR, but age, systolic blood pressure, serum level of uric acid, protein and cholesterol and GPX plasma activity with proteinuria. All these results showed that, in addition to several other well known risk factors for DN, products of oxidative lipid and protein damage are significant independent predictors of DN.

The authors declare no conflicts of interest.
Autori izjavljuju da nemaju sukob interesa.

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Oksidativni stres i drugi faktori rizika dijabetesne nefropatije u tipu 2 dijabetes melitusa

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Uvod. Cilj rada je bio da se ispita da li su biomarkeri oksidativnog stresa i antioksidantni enzimi faktori rizika za nastanak dijabetesne nefropatije (DN).

Metode. Studija je obuhvatila 70 bolesnika sa tipom 2 dijabetesa (37 muškaraca, starosti 41-81 godina) svrstanih u dvije grupe: prva od 32 bolesnika sa DN i druga sa 38 bolesnika bez DN. U ispitivanje oksidativnog stresa uključeno je i 15 zdravih osoba. Svim bolesnici uzeti su anamnestički podaci, urađen objektivni pregled i laboratorijske analize seruma i urina kojima je provjeren kvalitet glikoregulacije i funkcija bubrega. Proteinske tiol grupe (P-SH), antioksidantni enzimi [superoksid dizmutaza (SOD) i glutation peroksidasa (GPX)] određeni su u plazmi spektrofotometrijski, a malondialdehid (MDA) ELISA metodom.

Rezultati. Između dvije grupe bolesnika nije bilo razlike u demografskim karakteristikama, trajanju i metodama liječenja dijabetesa, krvnom pritisku, glikemiji našte i HbA1c. Bolesnici sa DN su imali veći indeks tjelesne mase (BMI), manju jačinu glomerulske filtracije (eGFR), a veću albuminuriju i proteinuriju. Aktivnost GPX i SOD u plazmi kao i koncentracija MDA i P-SH grupa nije se razlikovala između bolesnika dvije grupe, ali je aktivnost GPX i SOD bila značajno manja, a koncentracija MDA značajno veća kod bolesnika nego kod zdravih kontrola. Pol, starost, BMI, HbA1c i koncentracija P-SH i MDA u plazmi su izdvojeni kao značajni prediktori DN. Starost bolesnika, trajanje dijabetesa, koncentracija fosfora i mokraćne kiseline i aktivnost SOD u plazmi su negativno povezani sa eGFR. Starost bolesnika, koncentracija proteina i albumina u serumu, aktivnost GPX u plazmi su negativno, a sistolni krvni pritisak, koncentracija mokraćne kiseline i holesterola u serumu pozitivno povezani sa proteinurijom.

Zaključak. Biomarkeri oksidativnog oštećenja proteina i lipida izdvojeni su kao faktori rizika za DN pored nekoliko već dobro poznatih faktora rizika.

Ključne riječi: dijabetesna nefropatija, antioksidantni enzimi, biomarkeri oksidativnog stresa