

# Expression of mitochondrial superoxide dismutase in polymorphonuclear leukocytes from patients with type 1 diabetes with and without microvascular complications

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## KEY WORDS

expression of mitochondrial superoxide dismutase gene, microvascular complications, oxidative stress, type 1 diabetes

## ABSTRACT

**INTRODUCTION** One of the causes of impaired antioxidant response in patients with type 1 diabetes might be decreased expression of mitochondrial manganese superoxide dismutase (MnSOD).

**OBJECTIVES** The aim of this study was to evaluate the expression of MnSOD on transcript and protein levels in polymorphonuclear leukocytes (PMNLs) from patients with type 1 diabetes and analyze its association with microvascular complications.

**PATIENTS AND METHODS** The MnSOD expression was assessed in PMNLs from 46 patients with type 1 diabetes and 12 age- and sex-matched healthy subjects. The study group was divided into 2 subgroups: with and without microvascular complications. The MnSOD expression on the transcript level was evaluated by real-time quantitative polymerase chain reaction, while that on the protein level by Western blot analysis.

**RESULTS** A significant increase in the MnSOD transcript level was observed in all patients with diabetes with and without microvascular complications ( $P = 0.01$ ,  $P = 0.02$ , respectively). The MnSOD protein level was higher in patients without microvascular complications compared with those with complications and the control group ( $P = 0.05$ ,  $P = 0.03$ , respectively). The MnSOD expression was positively correlated with fasting plasma glucose and total cholesterol levels both at the transcript level ( $r = 0.4$ ,  $P < 0.05$  for both correlations) and at the protein level ( $r = 0.3$  and  $r = 0.4$ , respectively,  $P < 0.05$ ).

**CONCLUSIONS** Although an increased MnSOD transcript level in patients with type 1 diabetes suggests enhanced antioxidant mobilization in all diabetic patients, decreased levels of the MnSOD protein in PMNLs from patients with microvascular complications compared with those without complications indicates that patients with microvascular complications may have impaired antioxidant response.

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**INTRODUCTION** Despite considerable advances in the treatment of type 1 diabetes, chronic complications still remain the principal cause of morbidity and mortality in this patient group.<sup>1</sup> Recent clinical studies have shown that persistent

hyperglycemia (the main determinant of the development of chronic complications) coincides with oxidative stress.<sup>2,3</sup> Oxidative stress under diabetic conditions may result from increased superoxide production,<sup>4</sup> which causes the activation

of several signal pathways such as protein kinase C, c-Jun-N-terminal kinase, and p38 mitogen-activated protein kinase. This results in the dysfunction and apoptosis of endothelial cells.<sup>5-7</sup> Moreover, oxidative stress leads to overexpression of transcription factors, such as NF- $\kappa$ B, that are involved in the regulation of genes associated with the development of chronic inflammation.<sup>8</sup> All these pathological changes precede microangiopathy, including retinopathy, nephropathy, and neuropathy.<sup>9</sup> Therefore, there is growing evidence that the harmful effect of oxidative stress on endothelial cells has a pivotal role in the development and progression of chronic complications in patients with diabetes.<sup>10,11</sup> The level of reactive oxygen species (ROS) is regulated by a number of endogenous enzymes and exogenous antioxidants.<sup>12,13</sup> One of the most important antioxidant enzymes is mitochondrial manganese superoxide dismutase (MnSOD).<sup>14</sup> It is encoded by a nuclear gene and is transported from the cytoplasm into the mitochondrial matrix, where it catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide.<sup>15,16</sup>

Recent animal and clinical studies have revealed that the biological effect of MnSOD could be impaired in hyperglycemia.<sup>17,18</sup> It was observed that decreased transcript levels of MnSOD in patients with diabetes,<sup>19</sup> in bovine retinal endothelial cells treated with high glucose level, and in diabetic rats<sup>20</sup> were associated with enhanced oxidative stress and development of microangiopathy, i.e., nephropathy<sup>19</sup> and retinopathy.<sup>20</sup>

Apart from the endothelial cells, where the development of late diabetic complications is observed, polymorphonuclear leukocytes (PMNLs) are also damaged by oxidative stress. These cells are easily obtained but have disadvantages such as having fewer mitochondria and an invariably low degree of heteroplasmy caused by their rapid turnover. Nevertheless, the study of mitochondrial oxidative stress in PMNLs could indirectly reflect the events occurring in the tissues targeted by microvascular complications.<sup>21</sup> Therefore, the aim of this study was to evaluate the MnSOD expression both at transcript and protein levels in PMNLs from patients with type 1 diabetes and analyze its association with microvascular complications.

**PATIENTS AND METHODS** **Study group** The study was performed at the Department of Internal Medicine and Diabetology and Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poland. It was approved by the Ethics Committee of the Poznan University of Medical Sciences (No. 607/12), and written informed consent was obtained from all participants.

The study included 28 women and 18 men with type 1 diabetes. Diabetes was diagnosed in all patients according to the criteria of the American Diabetes Association on the basis of typical symptoms, blood glucose concentrations higher

than 11.1 mmol/l, and C-peptide concentrations lower than 0.5  $\mu$ g/l.<sup>22</sup> All patients were treated with intensive insulin therapy from the onset of the disease (mean daily basal insulin doses were  $0.37 \pm 0.05$  IU/kg). In addition, 10 patients were treated with angiotensin-converting enzyme inhibitors after the diagnosis of diabetic kidney disease.

At the time of the study, the mean age of the diabetic group was  $28 \pm 8$  years, and the mean diabetes duration was  $17 \pm 8$  years. In all patients, medical history was taken including the assessment of the body mass index (BMI), blood pressure, and lipid profile. In addition, all patients were screened for microvascular complications, i.e., diabetic retinopathy, diabetic kidney disease, and diabetic neuropathy.

Diabetic retinopathy was diagnosed by direct ophthalmoscopy through dilated pupils followed by fundus photography in all patients. It was graded according to the classification of the American Academy of Ophthalmology into no retinopathy, nonproliferative retinopathy (mild, moderate, and severe), and proliferative retinopathy.<sup>23</sup>

Diabetic kidney disease was detected at the stage of albuminuria (urinary albumin excretion rate between 30 and 300 mg/24 h) in 2 samples collected over a 3-month period after the exclusion of secondary causes of microproteinuria. Diabetic kidney disease was defined as the presence of albuminuria associated with diabetes lasting more than 10 years or with diagnosed diabetic retinopathy.<sup>24</sup>

Diabetic neuropathy was diagnosed if patients had 2 or more of the following: the presence of symptoms of neuropathy, absence of ankle tendon reflexes, and abnormal scores for pressure and/or vibration perception.<sup>22</sup>

Retinopathy was diagnosed in 19 patients, diabetic kidney disease in 10 patients, and neuropathy in 10 patients. Of these patients, 10 had only retinopathy, 1 had only diabetic kidney disease, 1 had only neuropathy, and 11 had more than 1 microvascular complication. Therefore, based on the presence or absence of microangiopathy, the study group was divided into 2 subgroups: patients without any microvascular complications (group 1) and patients with retinopathy or diabetic kidney disease or neuropathy or any combination thereof (group 2).

None of the subjects included in the study had chronic inflammatory diseases, such as atherosclerosis or rheumatoid arthritis, or infectious diseases.

The control group consisted of 12 age- and sex-matched healthy volunteers who underwent basic medical examination. They had normal BMI and levels of fasting glucose and total cholesterol (TC).

**Blood samples and polymorphonuclear leukocytes** Blood samples were collected between 8:00 and 10:00 a.m. (after 8-hour fasting) into tubes without an anticoagulant and tubes with the EDTA anticoagulant using a standard

venipuncture technique. To obtain serum, the samples were allowed to clot at room temperature and then centrifuged at  $2\,000 \times g$  for 15 min. Basic clinical assessment was performed on the day of sample collection. Five ml of blood in EDTA tubes was used for isolation of human PMNL by standard density gradient centrifugation<sup>25</sup> on Gradisol G (1.115 g/ml, Polfa, Poland). Briefly, 5 ml of blood was layered on 5 ml of Gradisol G in 15-ml plastic tubes and centrifuged for 45 min at  $400 \times g$ . The PMNL-rich pellet was collected into another 15-ml tube and washed twice with phosphate-buffered saline (PBS, Biomed, Poland) and centrifuged 15 min at  $400 \times g$  to remove the remaining Gradisol G. The obtained pellet of PMNLs was used for isolation of total RNA and proteins.

**Biochemical parameters** The analysis of biochemical parameters was done in an accredited medical laboratory. Serum concentrations of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and fasting plasma glucose (FPG) were determined using the commercially available assay kits (Roche Diagnostics GmbH, Germany). Low-density lipoprotein cholesterol (LDL-C) levels were obtained using the following formula:  $LDL-C = TC - HDL-C - TG/2.2$ . Postprandial plasma glycemia (PPG) was calculated as the mean of plasma concentration of glucose 2 h after 3 main meals in self-control from 3 days before recruitment. Hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) levels were measured using high-performance liquid chromatography with the Variant Hemoglobin A1c Program (Bio-Rad Laboratories, Hercules, California, United States).<sup>26</sup> The estimated glomerular filtration rate (eGFR) was measured using the Modification of Diet in Renal Diseases formula.

**Reverse transcription and real-time quantitative polymerase chain reaction analysis** Total RNA from PMNLs of the patients with type 1 diabetes and healthy subjects was isolated according to the method of Chomczynski and Sacchi.<sup>27</sup> The concentration and purity of RNA was determined by measuring the absorbance of RNA at 260-nm and 280-nm wavelengths (NanoDrop 200, Thermo Fisher Scientific, United States). RNA integrity was estimated by visual examination of 2 distinct RNA bands (28S and 18S) on 1% agarose gel stained with ethidium bromide. Complementary DNA (cDNA) was prepared using QuantiTect Reverse Transcription Kit (Qiagen, Germany). Real-time quantitative polymerase chain reaction (PCR) was performed in a Light Cyclers 480 real-time detection system (Roche Diagnostics GmbH, Germany) using SYBR® Green I as detection dye. For amplification, 1 µl of cDNA solution was added to 9 µl of Light Cyclers 480 SYBR Green I Master mix (5× concentrated) (Roche Diagnostics GmbH, Germany) and MnSOD forward and reverse primers. PCR amplification efficiency for target and reference genes was determined by standard curves created by

consecutive dilution of the cDNA template mixture, as described in the Relative Quantification Manual (Roche Diagnostics GmbH, Germany). The transcript level of target MnSOD gene was normalized to the reference peptidylprolyl isomerase A (PPIA) gene. MnSOD transcript levels in study subjects were expressed as multiplicity of cDNA concentrations in the calibrator. The specific primers used for *MnSOD* were 5'AAGGCTCAGGTTGGGGTTGG3' and 5'CTGAAGGTAGTAAGCGTGTCTC3', product size 149bp, and for *PPIA*; 5'TCCAGGGTTTATGTGTCTAG3' and 5'ATCCAACCACTCAGTCTTG3', product size 199bp.

**Western blot analysis** PMNLs were treated with lysis RIPA buffer (Sigma Aldrich, USA). Subsequently, 5 µl of protein sample were resuspended in loading buffer and separated on 12% Tris-glycine gel using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Gel proteins were transferred to a polyvinylidene fluoride membrane, which was blocked with 5% milk in Tris/HCl saline/Tween buffer. Immunodetection of bands was performed with mouse MnSOD monoclonal antibody (Abnova, Taiwan), followed by incubation with goat antimouse HRP-conjugated immunoglobulin G (IgG). To ensure equal protein loading, the membrane was restripped and incubated with anti-actin HRP-conjugated IgG (Santa Cruz, United States). Bands were revealed using the SuperSignal® West Femto Chemiluminescence substrate (Thermo Fisher Scientific, United States) and Biospectrum1 Imaging System 500 (UVP LTD, United States). MnSOD protein levels were presented as the ratio of MnSOD to β-actin band optical density.

**Statistical analysis** All results were expressed as mean ± standard deviation or median with interquartile range. Statistical hypotheses were tested using either the Kruskal–Wallis or Mann–Whitney test for variables lacking normal distribution, and the analysis of variance (ANOVA) or *t* test for normally distributed variables.

The ANOVA was used to assess the differences in age, BMI, systolic and diastolic blood pressure (SBP and DBP) between patients with and without microvascular complications and the control group. The Kruskal–Wallis test was performed to determine the differences in FPG and PPG levels, HbA<sub>1c</sub> levels, and parameters of lipid profile between the groups. The *t* test was used to compare diabetes duration between the 2 subgroups of patients with type 1 diabetes. The Mann–Whitney test was performed to detect the differences in the albumin-to-creatinine ratio between patients with and without diabetic complications and the differences of transcript and protein levels of MnSOD between the studied groups and male and female patients.

The Spearman correlation coefficient was used to test the strength of any associations between different variables in patients with type 1 diabetes and the control group. A *P* value of less than 0.05 was

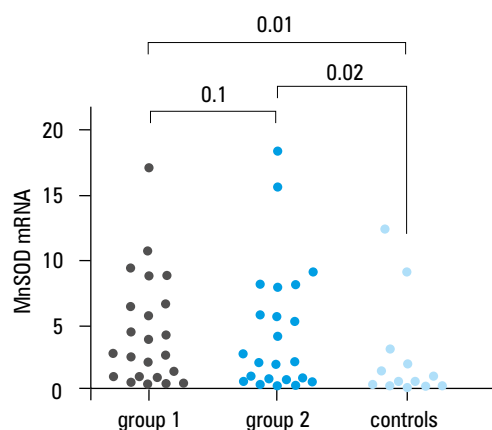
**TABLE** Basic characteristic of the patients with type 1 diabetes with and without microvascular complications and the control group

Characteristic	Type 1 diabetes		Control group (n = 12)	P value
	without microvascular complications (n = 23)	with microvascular complications (n = 23)		
sex (female/male)	18/5	10/13	7/5	–
age, y	30 ± 6	28 ± 8	31 ± 9	0.26 <sup>a</sup>
diabetes duration, y	15 ± 6	18 ± 6		0.69 <sup>b</sup>
BMI, kg/m <sup>2</sup>	25.1 ± 3.5	23.2 ± 2.2	22.8 ± 1.8	0.18 <sup>a</sup>
FPG, mmol/l	7.2 (6.9–8.5) <sup>e</sup>	8.1 (6.3–9.5) <sup>e</sup>	4.3 (4.1–4.6)	0.00001 <sup>c</sup>
PPG, mmol/l	8.1 (7.5–10.3) <sup>e</sup>	8.2 (6.9–10.1) <sup>e</sup>	5.2 (4.8–5.3)	0.00001 <sup>c</sup>
HbA <sub>1c</sub> , %	7.6 (6.9–8.2) <sup>e</sup>	7.5 (6.8–8.2) <sup>e</sup>	4.8 (4.3–5.5)	0.00003 <sup>c</sup>
TC, mmol/l	5.0 (4.4–5.7) <sup>e</sup>	5.1 (4.3–5.6) <sup>e</sup>	4.1 (4.0–4.5)	0.001 <sup>c</sup>
TG, mmol/l	0.9 (0.6–1.2)	1.0 (0.8–1.2)	1.0 (0.7–1.1)	0.36 <sup>c</sup>
LDL-C, mmol/l	2.8 (2.3–3.5) <sup>e</sup>	2.8 (2.5–3.5) <sup>e</sup>	2.3 (1.6–2.4)	0.001 <sup>c</sup>
HDL-C, mmol/l	1.5 (1.3–1.8)	1.6 (1.4–1.9)	1.3 (0.9–1.4)	0.4 <sup>c</sup>
SBP, mmHg	115 ± 10	118 ± 7	110 ± 10	0.56 <sup>a</sup>
DBP, mmHg	76 ± 6	77 ± 7	75 ± 6	0.5 <sup>a</sup>
albumin-to-creatinine ratio	2.1 (2.3–4.4) <sup>f</sup>	16.3 (3.0–45.4)	–	0.001 <sup>d</sup>
eGFR, ml/min/1.73 m <sup>2</sup>	81 (73–90)	80 (52–90)	–	0.97 <sup>d</sup>

Data are presented as mean ± standard deviation or median (range).

Abbreviations: BMI – body mass index, DBP – diastolic blood pressure, eGFR – estimated glomerular filtration rate, FPG – fasting plasma glucose, HbA<sub>1c</sub> – hemoglobin A<sub>1c</sub>, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, PPG – postprandial plasma glucose, SBP – systolic blood pressure, SD – standard deviation, TC – total cholesterol, TG – triglycerides

**a** analysis of variance; **b** *t* test; **c** Kruskal–Wallis test; **d** Mann–Whitney test; **e** statistical significance vs. the control group; **f** statistical significance vs. patients without microvascular complications



**FIGURE 1** Transcript level of mitochondrial superoxide dismutase (MnSOD) of polymorphonuclear leukocytes isolated from patients with type 1 diabetes without (group 1) and with (group 2) microvascular complications and the control group

considered statistically significant. The statistical analysis was performed with the STATISTICA 6.0 software (StatSoft, United States).

**RESULTS Clinical characteristics of the patients with type 1 diabetes** To identify the differences in the levels of basic clinical and biochemical variables that could be associated with the presence of microvascular complications, the 2 subgroups of diabetic patients were compared with

12 nondiabetic age- and sex-matched subjects in the control group.

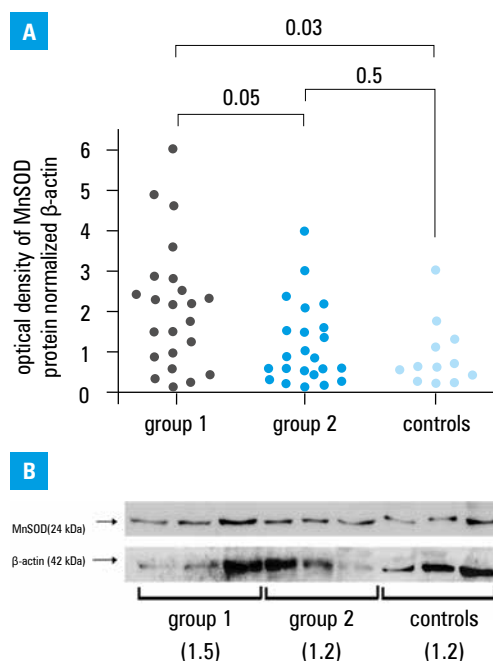
Diabetic patients did not differ from the control group in terms of BMI, SBP and DBP levels, as well as TG and HDL-C concentrations. Diabetic patients in both subgroups showed higher FPG and PPG levels, HbA<sub>1c</sub> value, and TC and LDL-C concentrations than the control group. The HbA<sub>1c</sub> value for most diabetic patients was not sufficient to maintain good metabolic control regardless of the presence or absence of microvascular complications. Patients with microvascular complications had a higher albumin-to-creatinine ratio than those without complications; on the other hand; there was no difference in eGFR levels between the subgroups (TABLE).

**Expression of mRNA and protein level of MnSOD in patients with type 1 diabetes** The transcript level of MnSOD was higher in PMNLs from diabetic patients without chronic complications compared with PMNLs from the control group (2.6 [0.8–6.4] and 0.6 [0.2–2.0], respectively; *P* = 0.01). It was also higher in PMNLs obtained from diabetic patients with chronic complications compared with PMNLs from the control group (2.2 [0.7–7.7] and 0.6 [0.2–2.0], respectively; *P* = 0.02). There was no difference in the transcript level of MnSOD between patients without and with microvascular complications (2.6 [0.8–6.4] and 0.6 [0.2–2.0], respectively; *P* = 0.1). Data are presented in FIGURE 1.



## FIGURE 2

**A** – transcript level of mitochondrial superoxide dismutase (MnSOD) of polymorphonuclear leukocytes isolated from patients with type 1 diabetes without (group 1) and with (group 2) microvascular complications and the control group; **B** – representative image of Western blot for 3 patients without chronic complications, 3 patients with chronic complications, and 3 healthy subjects



Interestingly, the level of the MnSOD protein was higher in PMNLs from patients without complications compared with those from patients with complications (1.7 [0.8–2.8] and 0.8 [0.4–1.6], respectively;  $P = 0.05$ ). It was also higher in patients without complications compared with the control group (1.7 [0.8–2.8] and 0.6 [0.3–1.3], respectively;  $P = 0.03$ ). There were no significant differences in MnSOD protein levels between PMNLs from patients with complications and those from the control group (0.8 [0.4–1.6] and 0.6 [0.3–1.3], respectively;  $P = 0.5$ ; **FIGURE 2**).

To assess the effect of sex on the expression of mRNA and protein levels of MnSOD, we compared male and female subgroups of diabetic patients. We did not observe any differences between men and women in the group without chronic complications (2.09 [1.038–5.999] and 1.96 [0.708–5.671], respectively,  $P = 0.4$ ) or in the group with chronic complications (1.011 [0.531–2.073] and 0.691 [0.489–2.393], respectively,  $P = 0.2$ ).

### Correlations between MnSOD expression and biochemical parameters in patients with type 1 diabetes

We also investigated the associations between biochemical parameters and the expression of MnSOD in PMNLs from patients with type 1 diabetes. The transcript level of MnSOD was positively correlated with FPG and TC concentrations ( $r = 0.4$ ,  $r = 0.39$ ,  $P < 0.05$ , respectively). Similar results were obtained for the MnSOD protein level ( $r = 0.31$ ,  $r = 0.39$ ,  $P < 0.05$ ). No correlations between biochemical parameters and the MnSOD expression in the control group were observed.

**DISCUSSION** Oxidative stress associated with diabetes may be caused by increased production of ROS or reduced capacities of antioxidant

enzymes.<sup>28,29</sup> Moreover, accumulating evidence demonstrates a link between oxidative stress and the development of chronic complications in patients with diabetes.<sup>10,30</sup> Possibly, a decreased expression of MnSOD may accelerate progression of chronic diabetic complications.<sup>18,19</sup> Therefore, the measurement of MnSOD expression at transcript and protein levels may help assess the risk of oxidative stress associated with diabetes.

In this study, we found an increased transcript level of MnSOD in PMNLs from patients with type 1 diabetes with and without chronic complications compared with the control group. To our knowledge, it is the first clinical study reporting such findings. In the studies by Ceriello et al.<sup>17</sup> and Hodgikson et al.,<sup>19</sup> an increased mRNA level of MnSOD was not observed; however, increased mRNA level and activity of other important antioxidant enzymes such as catalase (CAT) and glutathione peroxidase (GPx) were shown either in PMNLs<sup>19</sup> or in skin fibroblast<sup>17</sup> from patients with type 1 diabetes without chronic complications exposed to high glucose concentrations. Interestingly, both research groups observed failure of this defensive mechanism in the cells derived from patients with type 1 diabetes and with diagnosed nephropathy, showing that antioxidant response in patients with nephropathy is impaired.<sup>17,19</sup> In another study, a strong association between decreased mRNA levels of GPx, MnSOD, and CAT and development of retinopathy was observed in PMNLs from 67 patients with type 2 diabetes.<sup>29</sup> These results, in contrast to ours, indicate that antioxidant response may be decreased in diabetic patients with developed microangiopathy already at the transcript level. Therefore, we suggest that the inconsistency between their findings and ours may result from the limitation of the present study in terms of small study subgroups.

On the other hand, there are studies showing that diabetes is associated with an increased activity of antioxidant enzymes regardless of the presence or absence of chronic microvascular complications. However, this increased activity could not protect patients against the harmful effect of increased ROS levels caused by hyperglycemia.<sup>31,32</sup>

Although we did not find a significant difference for the MnSOD transcript level between the 2 subgroups of diabetic patients, we indicated higher levels of the MnSOD protein in PMNLs from patients without microvascular complications compared with those with complications and the control group. We hypothesize that a lower protein level of MnSOD in patients with microvascular complications compared with those without complications could result from enhanced glycation process of the MnSOD enzyme<sup>33</sup> and dramatically increased production of ROS caused by a pathological process associated with endothelial dysfunction, which occurs in patients with type 1 diabetes and chronic complications.<sup>34,35</sup> Unfortunately, we did not assess the glycation process

or measure oxidative stress in our study. Therefore, to confirm our preliminary results, further research is needed.

Nevertheless, our results show that impairment of functional antioxidant defense could be one of the reasons for the development and progress of chronic complications in type 1 diabetes, which is in line with studies showing a relation between the enhanced level of oxidative stress and development of chronic complications in patients with diabetes.<sup>30,36</sup> In a study assessing the relationship of retinal neuron apoptosis with MnSOD activity at an early phase of diabetic retinopathy, a decreased activity of MnSOD was observed with extended course of the disease in the retinas obtained from diabetic rats. It suggests that the longer the course of diabetes, the worse the oxidative damage of the retina and the lower the antioxidative capacity of MnSOD.<sup>37</sup> Moreover, our results are consistent with in-vitro and in-vivo findings showing a crucial role of antioxidant enzymes in protection against the development of chronic diabetic complications.<sup>38,39</sup> Overexpression of the MnSOD protein may inhibit progression of chronic diabetic complications in bovine endothelial cells and mice with induced diabetes.<sup>40-42</sup> It was found that an increased MnSOD expression in the endothelial cells may decrease ROS production caused by hyperglycemia. It leads to the inhibition of biochemical pathways involved in the pathogenesis of chronic complications: glucose-induced activation of protein kinase C, NF- $\kappa$ B pathway, and formation of advanced glycation end-products.<sup>40</sup> Moreover, in transgenic mice overexpressing MnSOD in endothelial cells, decreased transcript and protein levels of vascular endothelial growth factor and fibronectin in the retinas. Probably, it prevents the development of diabetic retinopathy in vivo.<sup>41</sup> Similar results were obtained in the study of Kowluru et al.,<sup>42</sup> in which the overexpression of MnSOD in diabetes-induced mice maintained a physiological level of glutathione, 8-hydroxy-2'-deoxyguanosine, and nitrotyrosine, which may inhibit the development of retinopathy.

Positive correlations between FPG and the expression of MnSOD observed in diabetic patients in our study could reflect an adaptive response to a pro-oxidant status caused by hyperglycemia. It is consistent with a study by Bémeur et al.,<sup>43</sup> who reported increased MnSOD protein expression under hyperglycemic conditions in rats. Interestingly, in contrast to our results, they did not observe a correlation between the MnSOD transcript level and glucose concentration. In addition, a positive correlation between TC concentrations and MnSOD expression was observed in our study. It suggests that not only the hyperglycemic status but also lipid metabolism abnormalities could contribute to increased antioxidant response in patients with type 1 diabetes.

In conclusion, even though increased MnSOD transcript levels in patients with type 1 diabetes

suggest enhanced antioxidant mobilization in all diabetic patients, a decreased level of MnSOD protein in PMNLs from patients with type 1 diabetes without microvascular complications compared with those with complications indicates that the latter may have impaired antioxidant response.

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# Ekspresja genu mitochondrialnej dysmutazy ponadtlenkowej w leukocytach polimorfojądrowych wyizolowanych od pacjentów z cukrzycą typu 1 z i bez rozpoznanych powikłań o charakterze mikroangiopatii

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## SŁOWA KLUCZOWE

cukrzyca typu 1,  
ekspresja genu  
mitochondrialnej  
dysmutazy  
ponadtlenkowej,  
mikroangiopatia, stres  
oksydacyjny

## STRESZCZENIE

**WPROWADZENIE** Jedną z przyczyn upośledzonej odpowiedzi antyoksydacyjnej u pacjentów z cukrzycą typu 1 może być zmniejszona ekspresja genu mitochondrialnej manganowej dysmutazy ponadtlenkowej (*manganese superoxide dismutase* – MnSOD).

**CELE** Celem badań była ocena ekspresji genu MnSOD na poziomie transkryptu i białka w leukocytach polimorfojądrowych (*polymorphonuclear leukocytes* – PMNL) wyizolowanych od pacjentów z cukrzycą typu 1 oraz analiza związku tych ekspresji z występowaniem powikłań o charakterze mikroangiopatii.

**PACJENCI I METODY** Ekspresja MnSOD była oceniana w PMNL wyizolowanych z krwi pobranej od 46 pacjentów z DM1 oraz dobranej wiekowo i pod względem płci 12 zdrowych osób. Grupa została podzielona na dwie podgrupy: z powikłaniami o charakterze mikroangiopatii lub bez takich powikłań. Ekspresja MnSOD na poziomie transkryptu była oceniana metodą PCR w czasie rzeczywistym (*real-time quantitative polymerase chain reaction*), natomiast ekspresja MnSOD na poziomie białka była mierzona za pomocą techniki Western blot.

**WYNIKI** Obserwowano zwiększoną ekspresję MnSOD na poziomie transkryptu u wszystkich pacjentów z cukrzycą zarówno z mikroangiopatią jak i bez niej (odpowiednio  $p = 0,01$ ;  $p = 0,02$ ). Poziom białka MnSOD był wyższy w grupie pacjentów bez mikroangiopatii w porównaniu z grupą pacjentów z mikroangiopatią oraz grupą kontrolną (odpowiednio  $p = 0,05$ ;  $p = 0,03$ ). U pacjentów z cukrzycą ekspresja MnSOD dodatkowo korelowała z poziomem glukozy na czczo oraz stężeniem cholesterolu całkowitego na poziomie transkryptu (dla obu korelacji  $r = 0,4$ ;  $p < 0,05$ ) oraz na poziomie białka (odpowiednio  $r = 0,3$  i  $r = 0,4$ ;  $p < 0,05$ ).

**WNIOSKI** Mimo że podwyższony poziom transkryptu MnSOD w grupie pacjentów z cukrzycą typu 1 sugeruje zwiększoną mobilizację antyoksydacyjną u wszystkich pacjentów z cukrzycą, to obniżony poziom białka MnSOD w komórkach PMNL u pacjentów z podgrupy bez mikroangiopatii w porównaniu z pacjentami z mikroangiopatią wskazuje, że pacjenci z przewlekłymi powikłaniami mogą mieć upośledzoną odpowiedź antyoksydacyjną.

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