Polymorphisms of superoxide dismutases and catalase and diabetes mellitus

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Abstract:
Background: Reactive oxygen species generated by hyperglycemia modify structure and function of lipids, proteins and other molecules taking part in chronic vascular changes in diabetes. Low activity of scavenger enzymes has been observed in diabetic patients and their protective role in oxidative stress may be deteriorated. This study was undertaken to investigate the association between polymorphisms of selected genes of antioxidant enzymes and diabetic macro- and microangiopathy.

Results: Significant differences in allele and genotype distribution among T1DM, T2DM and control persons were found in SOD1 and SOD2 genes but not in CAT gene, with p <0.01. Serum SOD activity was significantly decreased in T1DM and T2DM subjects compared to the control subjects with p<0.05. SOD1 and SOD2 polymorphisms affected SOD activity. Serum SOD activity was higher in CC than in TT genotype of SOD2 gene and higher in AA than in CC genotype of SOD1, both with p<0.05. Better diabetes control was found in patients with CC than with TT genotype of SOD2 gene. Significantly different allele and genotype frequencies of SOD2 gene polymorphism were found among diabetic patients with macroangiopathy and those without it. No difference was associated with microangiopathy in all genes.

Conclusions: The results of our study demonstrate that oxidative stress in diabetes mellitus can be accelerated not only due to increased production ROS by hyperglycemia but also by reduced ability of antioxidant defense caused at least partly by genetic polymorphisms of some scavenger enzymes.
Background

It is a well-established fact, that diabetes mellitus is a risk factor for cardiovascular disease [1]. Macrovascular complications of diabetes resulting in atherosclerotic cardiovascular disease such as coronary artery disease, cerebrovascular disease and peripheral vascular disease are leading causes of death in diabetic population [2]. It is also well-known fact that tight control of diabetes (measured by postprandial glucose and/or glycated haemoglobin) is effective in reducing clinical complications [3]. But not only optimal control of blood glucose level could prevent complications. One of the principal pathways to develop long-term vascular complications is the production of reactive oxygen species [4].

There are multiple sources of oxidative stress in diabetes including nonenzymatic, enzymatic and mitochondrial pathways [5]. ROS include free radicals such as superoxide (•O2-), hydroxyl (•OH), peroxyl (•RO2), hydroperoxyl (•HRO2-) as well as nonradical species such as hydrogen peroxide (H2O2) and hydrochlorous acid (HOCl). While ROS are generated under physiological conditions and are involved to some extent as signaling molecules and defense mechanisms as seen in phagocytosis, neutrophil function, and shear-stress induced vasorelaxation [6], excess generation of ROS has pathological consequences including damage to proteins, lipids and DNA [7]. ROS can stimulate oxidation of low-density lipoprotein, forming ox-LDL, which is not recognized by the LDL receptor. These can be taken up by scavenger receptors in macrophages leading to foam cell formation and atherosclerotic plaques [8].

ROS can activate several damaging pathways in diabetes including accelerated formation of advanced glycation end products [9], polyol pathway [10], hexosamine pathway and PKC [11], all of which have been proven to be involved in pathogenesis of micro- and macrovascular complications [12]. Under normal conditions, •O2- is quickly eliminated by antioxidant defense mechanisms. •O2- is dismutated to H2O2 by manganese superoxide
dismutase (MnSOD) in the mitochondria and by copper-zinc (CuZn-SOD) in the cytosol [13]. H2O2 is converted to H20 and O2 by glutathione peroxidase (GSH-Px) or catalase in the mitochondria and lysosomes, respectively [14]. H2O2 can also be converted to the highly reactive •OH radical in the presence of transition elements like iron and copper.

It still have to be elucidated the fact that in some patients are developed vascular complications in diabetes mellitus but this cannot be seen in the other patients with the same duration of diabetes mellitus and with the same level of disease control [15]. We consider genetic background may play role in this fact. In this study we have focused on genes coding antioxidant enzymes – superoxide dismutase and catalase, which are the leading enzymes in the defence against ROS.

Here we report a case-control study which examined the possible role of candidate polymorphisms in the genes encoding the antioxidant enzymes copper-zinc superoxide dismutase, manganese superoxide dismutase and catalase. Specifically, we have targeted single nucleotide polymorphisms for their likely functional role: SOD1 +35A/C (refSNP ID: rs2234694) which is located adjacent to a splice site (exon3/intron3 boundary), SOD2 Ala16Val (refSNP ID: rs4880) which has been suggested to alter protein structure [16] and function (C/T substitution in exon 2, codon position 2, aminoacid position 16) and catalase -21A/T (refSNP ID: rs7943316) which is located in the promotor region just proximal to the start site.

CuZn (copper-zinc) superoxide dismutase (CuZnSOD, also called SOD1, EC 1.15.1.1) is one of the cellular defense systems for oxidative insults. SOD1 is a cuproenzyme and catalyzes the disproportionation reaction of superoxide anion to oxygen and hydrogen peroxide at a bound copper ion [17]. SOD1 gene is located on chromozome 21q22, consisting of 5 exons. The increase of Cu, Zn-SOD expression in human smooth muscle cells protects against oxidant injury. OxLDL caused an increase in the DNA binding activity of activator protein-1
and nuclear factor kappaB, which is inhibited by Cu, Zn-SOD overexpression.

Overexpression of Cu, Zn-SOD attenuates the cell proliferation caused by oxLDL stimulation and that this inhibitory effect is mediated via downregulation of ERK1/2 and JNK phosphorylation and AP-1 and NF-kappaB inactivation.

Mn (manganese) superoxide dismutase (MnSOD, also called SOD2, EC 1.15.1.1) is present in mitochondria. The MnSOD gene is located on chromosome 6q25, consisting of 5 exons. C/T substitution (GCT/GTT) has been shown to change the structural conformation of the mitochondrial targeting sequence (MTS) of the enzyme. This substitution may lead to misdirected intracellular trafficking, followed by changes in enzyme activity in the mitochondria. Associations have been found between the Ala16Val SNP in the SOD2 gene and human neurodegenerative disorders as Parkinson's disease, tardive dyskinesia, sporadic motor neuron disease and nonfamilial idiopathic dilated cardiomyopathy, where also oxidative stress could play an important role in the pathogenesis [18].

Catalase (CAT), (EC 1.11.1.6), present in the peroxisomes of nearly all aerobic cells, serves to protect the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of ROS. The protein exists as a dumbbell-shaped tetramer of four identical subunits. Each monomer contains a heme prosthetic group at the catalytic center. The gene for human catalase has been mapped to chromosome 11p13. The catalase gene contains 13 exons, several SNPs in the gene have been reported, most of which are associated with acatalasaemia, an autosomal recessive trait characterized by a level of erythrocyte catalase which is only 0.2-4 % of normal catalase activity [19]. In this study we focused on a common A/T polymorphism located at –21 A/T.

**Methods**
Subjects
Total of 120 Type 1 (T1DM), 306 Type 2 (T2DM) diabetic patients and control group of 140 healthy subjects without family history of diabetes were examined in this study.
Microangiopathy was confirmed by ophthalmoscopy or by the presence of peripheral neuropathy (diagnose was based on clinical features and by physical examination by 10 g monofilament, tuning fork and biothesiometry) in 167 patients who did not have any evidence of macrovascular disease from the clinical picture (no history of angina pectoris, normal ECG records or normal coronarogram). In case of suspicion on autonomic neuropathy made from physical examination (tachycardia recorded by ECG in resting state, systolic blood pressure reaction on orthostatism) patients were excluded from this group. 66 subjects had macrovascular complications manifested by ischemic heart disease (diagnosis was based on ECG or coronarography), ischemic disease of the lower limbs (diagnosis was based on angiography of lower limbs arteries) or had history of stroke (diagnosis based on clinical features and CT). The remaining 161 diabetic patients were free of any complications. Clinical and laboratory characteristics are shown in Tab.1.

Laboratory measurements
Venous blood samples were drawn after an overnight fast. Plasma (Li-heparine) glucose, creatinine were measured in central biochemistry laboratory. Serum total cholesterol, HDL-cholesterol and triglycerides (TG) were measured by automated enzymatic methods on Hitachi analyzer, LDL cholesterol was calculated according to Friedwalds formula. HbA1c was measured by high-performance liquid chromatography. Superoxid dismutase activity was determined spectrophotometrically by xanthine/xanthine oxidase system by Genesys 5 spectrophotometer, USA. The method is based on the well-known reaction described by McCord and Fridovich [20]. SOD activity was expressed in international units (U).
**DNA analysis**

Blood was extracted from the peripheral blood (5-10 ml) and genomic DNA was prepared from leucocytes (minimal amount of leucocytes was $3.5 \times 10^9/l$) by sodium dodecyl sulphate (SDS) lysis by ammonium acetate extraction and ethanol precipitation. Determination of the SOD and CAT polymorphisms was achieved by polymerase chain reaction followed by restriction digestion. Details of each genotyping assay are shown in Tab.2. Digested PCR products were visualised by ultraviolet transillumination following ethidium bromide staining and migration compared against DNA ladder and a positive RFLP control sample.

**Statistical analysis**

Age, BMI and duration of diabetes were compared between studied groups using Student's $t$-test. Statistical analyses of frequency counts were performed using the Chi-square ($\chi^2$) test. Comparison of continuous variables (HbA1c) among the SOD genotypes was performed with the use of analysis of variance (ANOVA). A logistic regression analysis was performed to evaluate the interaction between the genotypes and other variables in relation to the prevalence of macro- or microangiopathy. In this analysis, the dependent variable was the presence or absence of vascular complication. Independent variables included in this analysis were BMI, age, present HbA1c level, type of diabetes, duration of diabetes, PON1 activity and PON1 genotype. P values $<0.05$ were considered as significant. The laboratory data are expressed as means ± S.D. The analysis was performed using programme Statistica 6.0 (StatSoft).

**Results**

**SOD activity**
Serum SOD activity was significantly decreased in T1DM (0.75±0.18 U; 95% CI: 0.72, 0.79) and in T2DM patients (0.71±0.33; 95% CI: 0.67, 0.74) compared to the control subjects (1.67±0.33, 95% CI: 1.61, 1.72), both p< 0.01. Differences between T1 and T2 in SOD activity were not found statistically significant (p=0.14). No gender or age influence on its activity was found in diabetic or healthy subjects. The lower serum SOD activity was found in patients (T1 and T2) with macrovascular (0.51±0.31 U; 95%CI: 0.43, 0.58) than in those with microvascular complications (0.74±0.16 U; 95%CI: 0.71, 0.76) or without any vascular complications (0.76± 0.34 U;95%CI: 0.71, 0.81), both with p< 0.01.

**Allele and genotype frequency comparisons**

*The effect of the SOD1 +35A/C polymorphism on SOD activity in healthy subjects and diabetic patients with DM.*

The AA genotype was the most common in healthy subjects followed by the AC genotype, whereas the AC was more common than the AA genotype in T1DM and T2DM patients (Tab. 3). Significant differences between the allele and genotype frequencies (Tab.3) for the SOD1 +35A/C polymorphism was observed in T1DM as compared to controls (A: 0.69 vs 0.52, p<0.01; C: 0.31 vs. 0.48, p<0.05) and similarly in T2DM (A: 0.58 vs. 0.52; C: 0.42 vs 0.48, p<0.05). This SNP was related to SOD serum activity. Higher activities were found in AA than in CC genotypes of diabetic patients but not in control subjects (Tab. 3).

*Relationship between the SOD2 Ala16Val (C/T) polymorphism and SOD activity in healthy subjects and patients with DM.*

The TT genotype (Val/Val) was the most common in both T1DM and T2DM patients, CT genotype was the most common in healthy subjects whereas the CC genotype (Ala/Ala) was the rarest one in all groups (Tab. 3). The allele frequency of the SOD2 polymorphisms was significantly different in healthy persons compared to T1DM and T2DM patients (T allele
(Val): 0.54 (controls) vs. 0.81 (T1) or 0.85 (T2), p<0.05 and C allele (Ala): 0.46 (controls) vs. 0.19 (T1) or 0.15 (T2), p<0.05 (Fig. 1).

In all groups of diabetic patients SOD activity was the highest in the CC genotype (Ala/Ala) and the lowest in the TT genotype (Val/Val) (Tab. 3.).

**CAT polymorphism in diabetes mellitus.**

We found no statistically significant differences between frequencies in alleles of SNP between DM patients and healthy subjects (Tab.3). Control of diabetes was not influenced by polymorphisms in the CAT gene (Tab. 4).

*The association of SOD activity and SNPs in SOD1 and SOD2 gene with diabetes control and SOD1, SOD2 and CAT gene with vascular complications.*

In T1DM and T2DM patients diabetes control expressed by glycated haemoglobin values was poorer in TT genotype (Val/Val) of SOD2 (7,10±1,51; 95%CI: 6,42-7,91 in T1DM and 7,29±1,49; 95%CI: 6,70-8,46 in T2DM) than in CC genotype (Ala/Ala) of SOD2 (6,39±1,1; 95%CI: 5,7-7,0 in T1DM and 6,71±1,21; 95%CI: 5,73-6,99), p<0,05. No effect of SNP in SOD1 and CAT gene on diabetes control was found. Glycated haemoglobin was in AA genotype of SOD1 (6,7±1,4; 95%CI: 5,41-6,23 in T1DM and 6,9±1,4; 95%CI: 5,48-6,09 in T2DM) and in CC genotype of SOD1 (6,59±1,22, 95%CI: 5,08-6,24 in T1DM and 6,61±1,51; 95%CI: 5,02-6,05 in T2DM), p=0,124. Similar foundings were made in CAT gene, glycated haemoglobin in AA genotype of CAT (6,14±1,11; 95% CI: 6,06-6,57 in T1DM and 6,50±0,85; 95%CI: 6,32-7,25 in T2DM) and glycated haemoglobin in TT genotype of CAT (6,19±1,32, 95% CI: 6,09-6,60 in T1DM and 6,61±0,54; 95%CI: 6,45-7,05 in T2DM), p=0,249.

Significantly different genotype frequencies of SNPs in SOD1 and SOD2 genes were found in diabetic patients (T1DM and T2DM) with macroangiopathy (MA+). When compared these with CC genotype vs.AC and AA genotypes of SOD1: OR (odds ratio) 1.73; 95% CI
1.45-5.37 with p<0.05, CC genotype (Ala/Ala) vs. CT (Ala/Val) and TT (Val/Val) genotypes of SOD2: OR 0.62; 95%CI 0.58-0.90 with p<0.01. No association was found in CAT gene. When compared AA genotype vs. AT and TT genotypes of CAT, OR was 1.05; 95%CI 0.78-1.13, p=0.851. Macroangiopathy was associated with significantly higher frequency of C allele in SOD1 gene (0.58 in MA group vs. 0.42 in DM group without complications, p<0.01), lower frequency of C allele (Ala) in SOD2 gene (0.28 in MA group vs. 0.39 in DM group without vascular complications (MA-MI-), p<0.05) whereas no such distribution was found in CAT gene.

No differences in genotype frequencies were associated with microangiopathy (MI+).

When compared these with CC genotype vs. AC and AA genotypes in SOD1: OR 0.91; 95%CI 0.74-1.32 with p= 0.783, CC genotype (Ala/Ala) vs. CT (Ala/Val) and TT (Val/Val) genotypes in SOD2: OR 0.96; 95% CI 0.52-1.38 with p= 0.852 and AA genotype vs. AT and TT genotypes in CAT: OR 1.04 95%; CI 0.37-1.26 with p=0.814. No statistically significant distribution in allele frequencies was found in SNPs of all studied genes in microangiopathy (C allele in SOD1 was 0.47 in MI group vs. 0.42 in DM group without complications with p=0.118, T allele in SOD2 was 0.35 in MI group vs. 0.39 in DM group without complications with p= 0.242). Frequencies of genotypes ranged according to presence of vascular complications in both types of diabetes mellitus are showed in Fig. 1.

We found also negative correlation between serum superoxid dismutase activity (SOD) in both types of diabetes mellitus and the values of glycated haemoglobin (HbA1c %) (Fig. 2), as well as the presence of vascular complications in both types of diabetes (Fig.3).

Association of the SOD1, SOD2 and CAT polymorphism, BMI, age, duration of diabetes, sex, type of diabetes and SOD activity as independent variables with the presence of micro- or macroangiopathy as dependent variable was performed using a logistic regression model. This analysis indicated that SOD1 and SOD2 genotypes are significantly associated
with macroangiopathy. Another variables significantly associated (p<0.05) with angiopathy were HbA1c, SOD activity and duration of diabetes. No independent contribution has been demonstrated for age, sex, BMI and type of diabetes. (Tab.5).

**Discussion and conclusions**

In present study we found significantly different proportion of alelle and genotype distribution for studied SNPs of SOD1 and SOD2 genes in Type 1 and Type 2 diabetic patients as compared with healthy subjects. We found no significantly different proportion of allele and genotype distribution in CAT gene polymorphism. Our findings in SOD2 gene are in agreement with previous observation of other authors [21]. We also confirmed that serum SOD activity is significantly reduced in diabetic patients [22]. The presence of CC (Ala/Ala) genotype in SOD2 gene was associated with poorer diabetes control (expressed by glycated haemoglobin) than CT (Ala/Val) and TT (Val/Val) genotypes. Macroangiopathy was associated with significantly higher frequency of C allele of +35 A/C SNP of SOD1 gene and lower frequency of T (Val) allele of Ala16Val SNP of SOD2 gene whereas no such distribution was found in microangiopathy. Finally, we found negative correlation between SOD activity in both types of DM and level of control (expressed by glycated haemoglobin) and presence of micro- or macroangiopathy.

The polymorphisms of the key antioxidative enzymes could be among the factors that explain the high prevalence of macroangiopathy in patients with diabetes mellitus.

Functional polymorphism in the signal sequence of SOD2 (Ala16Val) has been identified in humans and appears to be a minor determinant of carotid atherosclerosis as demonstrated by increased carotid intima-media thickness [19]. The Ala/Val variation in the SOD2 leader signal affects the processing efficiency of the enzyme. The Ala type of SOD2 might have an alfa-helical structure that is a common conformation of mitochondrial leader signals, while the Val type might change its conformation from alfa-helix to beta-sheet with a substitution at
position 16 [23]. The alfa-helical structure is important for the effective transport of precursor proteins into mitochondria. The Val type is less efficiently transported into mitochondria than the Ala type of the enzyme. The processing study of these 2 leader signals has suggested that the basal level of the SOD2 activity might be highest for Ala/Ala (C/C), followed by Ala/Val (C/T) and then Val/Val (T/T).31 The Val variant of the SOD2 might be present at a lower concentration in mitochondria, and homozygous Val/Val [24]. Therefore, the observed positive association of macroangiopathy, worse diabetes control (high levels of glycated haemoglobin) with the SOD2-Val/Val genotype could be explained, at least in part, by the Val isoform of the SOD2 leader signal itself has lower resistance against ROS produced in mitochondria due to enzyme’s less efficient mitochondrial transport. This may lead to protein oxidation, mitochondrial DNA mutations and damage, common in the pathogenesis of late diabetic complications [25]. The Ala allele of the SOD2 gene is more widespread than the Val allele in healthy white populations. In contrast, the frequency of the Ala variant is significantly lower in Asian populations than in most white populations and the Val/Val genotype is most common in Asian populations.

We found similar effect on enzyme activity as in Ala16Val SNP of SOD2 also in A/C SNP of SOD1 gene. There is no evidence describing the way how the structure of CuZn SOD may be affected by this SNP. It is known that deficiency in CuZn-SOD results in increased levels of vascular superoxide and peroxynitrite, increased myogenic tone, augmented vasoconstrictor responses, and impaired endothelium-dependent (NO-mediated) relaxation in both large arteries and microvessels [26]. Alterations in expression of CuZn-SOD may also impact vascular structure. Evidence suggests that deficiency in CuZn-SOD produces hypertrophy of cerebral arterioles [26].

We found no statistically significant differences in distribution of CAT alleles in studied SNP and no impact on presence of vascular complications of DM, on level of glycated
haemoglobin. Hypocatalasemic patients were found to have higher plasma levels of homocysteine and lower levels of folate [19], suggesting that these patients are at greater risk for cardiovascular disease. Common functional polymorphisms in the catalase promoter have been identified in a Swedish population [27], although their relationship to vascular disease risk has not been determined. Interestingly, in an isolated Chinese population, a variant within the catalase promoter region has been associated with essential hypertension [28].

The study inconsistency in the association between genotypes and DM or cardiovascular disease is partly due to limits of conventional cross-sectional and retrospective case-control studies because selection bias have to be considered and the statistical analysis might have failed to demonstrate any significant differences. Large differences between ethnic populations are known in studied genotypes distribution which may be the reason for differences among studies.

In conclusion, the present findings show that the genotype distributions of the SOD1 and SOD2 in patients with both types of diabetes mellitus differed to those in nondiabetic individuals. Genetic background may be at least partly associated with diabetes control and consequently enzyme activities protecting against oxidative stress. Vascular disorders like atherosclerosis are then the results of combined genetic and metabolic changes.

**List of abbreviations**

- **T1DM**  
  Type 1 Diabetes Mellitus
- **T2DM**  
  Type 2 Diabetes Mellitus
- **ROS**  
  Reactive oxygen species
- **LDL**  
  low density lipoprotein
- **HDL**  
  high density lipoprotein
- **TG**  
  triacylglycerol
- **AGE**  
  advanced glycation endproduct
- **PKC**  
  protein kinase C
- **AP-1**  
  activated protein 1
- **NF-κB**  
  nuclear factor kappa B
- **ERK ½**  
  extracellular signal-regulated kinase
- **JNK**  
  c-Jun N-terminal kinase
- **SOD**  
  superoxide dismutase
Authors’ contributions

MF drafted the manuscript, participated in design of the study, performed the statistical analysis, carried out the molecular genetic, participated in the sequence alignment.
JS conceived of the study, participated in its design and coordination and helped to draft the manuscript.
JH carried out the enzyme activities assessment.
ZL carried out the molecular genetic, participated in the sequence alignment.
MJ helped with the coordination of the study, participated in the molecular genetic part of the study.
All authors read and approved the final manuscript.

Acknowledgements

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References


**Figure legends**

**Figure 1** Genotype frequencies according to presence of vascular complications.

Distribution of genotypes in SOD1 (A/C allele) and SOD2 (C/T allele, Ala/Val aminoacid) in both types of diabetes mellitus according to presence macroangiopathy (MA+) or microangiopathy (MI+) or diabetes mellitus with no complications (MA-MI-). Explanation of results is mentioned in the text.

**Figure 2** Correlation between SOD activity and glycated haemoglobin.

Data correlation between the values of glycated haemoglobin (HbA1c %) and serum superoxide dismutase activity (SOD) in both types of diabetes mellitus. The correlation coefficients (Spearman) are r1= -0.41 (T1DM), r2= -0.23 (T2DM) with p <0,05 . Dotted lines mean 95% confidence intervals.

**Figure 3** Correlation between presence of vascular complications and SOD activity.

Cross-correlation between the presence of vascular complications in diabetic patients and the level of serum superoxide dismutase activity. The correlation coefficients (Spearman) are r1= -0.29 (T1DM), r2= -0.28 (T2DM) with p<0,05. Dotted lines mean 95% confidence intervals.
### Tables

#### Table 1  Clinical and laboratory characteristics.

<table>
<thead>
<tr>
<th></th>
<th>T1DM</th>
<th>P values (a)</th>
<th>T2DM</th>
<th>Controls</th>
<th>P values (b)</th>
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<tbody>
<tr>
<td>Gender (males/females)</td>
<td>58/62</td>
<td>0.602</td>
<td>156/150</td>
<td>93/87</td>
<td>0.198</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>40±12</td>
<td><strong>0.022</strong></td>
<td>57±15</td>
<td>39±9</td>
<td><strong>0.043</strong></td>
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<tr>
<td>Duration of DM (years)</td>
<td>18±9</td>
<td>0.853</td>
<td>17±8</td>
<td>0</td>
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<td>BMI (kg/m2)</td>
<td>22±4</td>
<td><strong>0.03</strong></td>
<td>30±5</td>
<td>21±5</td>
<td><strong>0.027</strong></td>
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<td>Systolic BP (mmHg)</td>
<td>120±10</td>
<td>0.748</td>
<td>125±25</td>
<td>120±20</td>
<td>0.676</td>
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<tr>
<td>Diastolic BP (mmHg)</td>
<td>60±20</td>
<td>0.521</td>
<td>70±30</td>
<td>70±15</td>
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<td>Microvascular complications(n)</td>
<td>40</td>
<td><strong>0.048</strong></td>
<td>159</td>
<td>0</td>
<td>-</td>
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<td>Macrovascular complications(n)</td>
<td>14</td>
<td><strong>0.016</strong></td>
<td>52</td>
<td>0</td>
<td>-</td>
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<td>FPG (mmol/l)</td>
<td>6.60±1.35</td>
<td>0.058</td>
<td>7.82±2.29</td>
<td>4.95±0.76</td>
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<td>HbA1c (%)</td>
<td>6.1±1.9</td>
<td>0.321</td>
<td>6.7±1.8</td>
<td>0</td>
<td>-</td>
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<tr>
<td>GFR (MDRD) (ml/s/1.73m$^2$)</td>
<td>1.23±0.35</td>
<td>0.179</td>
<td>1.09±0.28</td>
<td>1.35±0.22</td>
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<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.8±0.5</td>
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<td>5.3±0.7</td>
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<td>HDL-C (mmol/l)</td>
<td>1.55±0.35</td>
<td>0.216</td>
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<td>LDL-C (mmol/l)</td>
<td>3.22±0.53</td>
<td>0.116</td>
<td>3.57±0.81</td>
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<td>Triglycerides (mmol/l)</td>
<td>1.31±0.30</td>
<td><strong>0.031</strong></td>
<td>1.99±0.79</td>
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Table 2  Sequences of used primers and used restrictases.

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<tr>
<td>SOD1</td>
<td>5´CTATCCAGAAAAACACGGTGCC 3´</td>
<td>Hhal</td>
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<tr>
<td>SOD2 A16V (C/T)</td>
<td>5´TGGTACTTCTCTCGTGACG3´</td>
<td>Bsawl</td>
</tr>
<tr>
<td>CAT -21 A/T</td>
<td>5´-AATCAGAAGGCAGTCCTCCC-3'</td>
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Table 3  SOD activities according to genotype, genotypes frequencies.

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<th>AC</th>
<th>CC</th>
<th>TT (Val/Val)</th>
<th>CT (Ala/Val)</th>
<th>CC (Ala/Ala)</th>
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<th>AT</th>
<th>TT</th>
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</thead>
<tbody>
<tr>
<td>T1DM</td>
<td>58 (48)</td>
<td>50 (42)</td>
<td>12 (10)</td>
<td>79 (66)</td>
<td>36 (30)</td>
<td>5 (4)</td>
<td>48(40)</td>
<td>53(44)</td>
<td>19(16)</td>
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<td></td>
<td>0.76±0.15</td>
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<tr>
<td>T2DM</td>
<td>104 (34)</td>
<td>147(48)</td>
<td>55(18)</td>
<td>220(72)</td>
<td>80(28)</td>
<td>6 (2)</td>
<td>110(36)</td>
<td>147(48)</td>
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<td></td>
<td>0.73±0.29</td>
<td>0.72±0.36</td>
<td>0.70±0.31</td>
<td>0.72±0.28</td>
<td>0.72±0.19</td>
<td>0.75±0.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>49 (27)</td>
<td>90 (50)</td>
<td>41(23)</td>
<td>52(29)</td>
<td>90 (50)</td>
<td>38(21)</td>
<td>67(37)</td>
<td>86(48)</td>
<td>27 (15)</td>
</tr>
<tr>
<td></td>
<td>1.66±0.31</td>
<td>1.67±0.35</td>
<td>1.66±0.28</td>
<td>1.58±0.28</td>
<td>1.60±0.35</td>
<td>1.63±0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The occurrence of genotypes in studied polymorphisms among T1, T2 diabetic patients and healthy subjects (controls), serum superoxide dismutase activity separated according to the genotypes in compared groups (AA/AC/CC in SOD1 gene, TT/CT/CC in SOD2 gene, AA/AT/TT in CAT gene). Data are expressed in mean±SD, n means number of cases, brackets mean genotype frequencies (allele frequencies and differences among them are mentioned in text), SOD act means enzyme activity.

Table 4  Genotype frequencies in CAT gene according to presence of vascular complications and values of glycated haemoglobin according to genotype in CAT gene.
Genotype frequencies of CAT gene according to the presence of vascular complications in patients with diabetes mellitus. *MA+* means presence of macroangiopathy, *MI+* means presence of microangiopathy, *MA-MI-* group involves patients with no vascular complications. *HbA1c* is glycated haemoglobin (%) marked as mean±SD.

Table 5  Logistic regression analysis for risk factors of vascular complications in diabetes mellitus.

<table>
<thead>
<tr>
<th>Variable</th>
<th>p (MA)</th>
<th>OR; 95%CI (MA)</th>
<th>p (MI)</th>
<th>OR; 95%CI (MI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD1 35 A/C</td>
<td>0,048</td>
<td>1,73; 1,45-5,37</td>
<td>0,783</td>
<td>0,91; 0,74-1,32</td>
</tr>
<tr>
<td>SOD2 A16V</td>
<td>0,009</td>
<td>0,62; 0,58-0,90</td>
<td>0,852</td>
<td>0,96; 0,52-1,38</td>
</tr>
<tr>
<td>CAT -21 A/T</td>
<td>0,851</td>
<td>1,05; 0,78-1,13</td>
<td>0,814</td>
<td>1,04; 0,37-1,26</td>
</tr>
<tr>
<td>SOD activity</td>
<td>0,040</td>
<td>0,48; 0,25-0,9</td>
<td>0,048</td>
<td>0,62; 0,44-0,91</td>
</tr>
<tr>
<td>Present HbA1c</td>
<td>0,039</td>
<td>1,28; 1,12-1,87</td>
<td>0,041</td>
<td>1,26; 1,12-1,81</td>
</tr>
<tr>
<td>BMI</td>
<td>0,686</td>
<td>0,97; 0,90-1,11</td>
<td>0,852</td>
<td>0,98; 0,87-1,04</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>0,038</td>
<td>1,96; 1,38-3,27</td>
<td>0,038</td>
<td>2,01; 1,50-4,31</td>
</tr>
<tr>
<td>Sex</td>
<td>0,86</td>
<td>0,97; 0,89-1,22</td>
<td>0,80</td>
<td>0,95; 0,88-1,15</td>
</tr>
<tr>
<td>Age</td>
<td>0,124</td>
<td>0,73; 0,49-1,54</td>
<td>0,152</td>
<td>0,81; 0,52-1,28</td>
</tr>
<tr>
<td>Type of diabetes</td>
<td>0,084</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*MA in brackets* means presence of macroangiopathy, *MI in brackets* means presence of microangiopathy, *OR* means odds ratio, *95%CI* means confidence interval (α=0,05). MA and MI are dependent variables. Genotype (SOD1, SOD2, CAT), SOD activity, HbA1c, duration
of diabetes, BMI, sex, age and type of diabetes act as independent variables. Variables significantly associated with macro- or microangiopathy are marked in table as bold.
Figure 3

Graphs showing the SOD activity (U) in T1DM and T2DM with respect to the presence of vascular complications (no complications, microangiopathy, macroangiopathy). The graphs indicate a decreasing trend in SOD activity with increased vascular complications in both T1DM and T2DM groups.