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Genetic polymorphisms (Pro197Leu of Gpx1, +35A/C of SOD1, −262C/T of CAT), the level of antioxidant proteins (GPx1, SOD1, CAT) and the risk of distal symmetric polyneuropathy in Polish patients with type 2 diabetes mellitus

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ABSTRACT

Purpose: Oxidative stress and impaired anti-oxidant defense are regarded as contributory factors for distal symmetric polyneuropathy (DSPN). The purpose of the study was to evaluate the plasma level of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) and the association between polymorphic variants in genes encoding for *GPx1*, *SOD*, *CAT* and the risk of DSPN in T2DM patients.

Material/methods: We included 401 individuals: 110 T2DM patients with DSPN, 135 T2DM patients without DSPN, and 156 control subjects with normoglycemia, and without DSPN. We employed RFPL-PCR to genotype polymorphic variants *Pro197Leu* of *Gpx1*, *+35A/C* of *SOD1*, *−262C/T* of *CAT* and ELISA tests to measure plasma level of SOD1, GPx1 and CAT. The odds ratios (ORs) and 95% confidence intervals (CIs) for each genotype and allele were calculated.

Results: There was a significant decrease in the level of GPx1 ($p < 0.05$), SOD1 ($p < 0.05$) in T2DM patients with DSPN compared to healthy subjects. T2DM patients without DSPN showed a statistically lower serum level of GPX1 ($p < 0.05$) than healthy subjects. SOD 1 and CAT levels were lower in T2DM patients with DSPN compared to T2DM patients without DSPN ($p < 0.05$). The genetic analysis revealed the lack of association between examined polymorphic variants and the risk of DSPN.

Conclusions: The examined polymorphic variants are not associated with DSPN in Polish T2DM patients. The obtained results suggest that disturbances in antioxidant defense system may play significant role in the development and progression of DSPN.

1. Introduction

Distal symmetric polyneuropathy (DSPN) is the most common, chronic complication of diabetes, affecting up to 90% of patients with type 2 diabetes mellitus (T2DM). It predisposes to severe functional limitations and serious complications including leg amputation [1]. The ethiology of DSPN in diabetic patients is still to be elucidated. It seems that increased oxidative stress and/or impaired anti-oxidant defense associated with diabetes may be associated with its development and progression, but the data on this topic is still scarce.

Chronic hyperglycemia is thought to be involved in the development of mitochondrial dysfunction that leads to reactive oxygen species (ROS) overproduction [2]. In diabetes additional sources of ROS, including glucose autoxidation, protein kinase C activation, methylglyoxal formation and glycation, hexosamine metabolism, and sorbitol formation increase oxidative stress [2–4].

Antioxidant defense system consisting of endogenous and exogenous antioxidants is responsible for protection against ROS. It is well documented that in (T2DM) the major antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) have an altered activity [5,6]. It is suggested that single nucleotide polymorphisms (SNPs)

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in genes coding for the antioxidant enzymes may lead to decreased or impaired ROS detoxification and increase oxidative damage. [7]. Therefore, it seems that genetic variations of antioxidant enzymes may influence the risk of chronic complications of diabetes, including DSPN [8,9].

GPxs are members of antioxidant defense system. They are responsible for reducing of hydrogen peroxide and great range of organic peroxidises to water with reduced glutathione. *GPx1* is the most common isoform of *GPx* family, presented in the cytoplasm of nearly all tissues. GPx1 is a tetrameric cytosolic protein consisting of four identical subunits, each containing one selenocysteine residue. *GPx1* gene contains 2 exons. A transition of C to T at nucleotide 594 in exon 2 corresponds to an amino acid change from Pro to Leu at codon 197 [10]. It was demonstrated that Leu allele was less responsive to stimulation of GPx1 by selenium supplementation than Pro allele [11]. It was also demonstrated that Leu allele may play a crucial role in determining genetic susceptibility to coronary-arteriosclerosis in T2DM [12].

SOD is a member of family that scavenges superoxide anions. SOD1 is copper-zinc superoxide dismutase located in the cytosol and in the mitochondrial intermembrane space. SOD1 probably plays the most important role because it represents between 50% and 80% of the total SOD activity [13]. *SOD1* gene is made up of five exons and the +35A/C polymorphism is adjacent to the slicing point (exon3/intron3) [14]. It was found that AA genotype of this polymorphism is associated with higher SOD1 activity [7]. SOD1 may be a candidate enzyme in diabetic neuropathy because SOD1-deficient mice developed motor axon degeneration due to mitochondrial damage. Fischer et al. found that targeted replacement of SOD1 in the mitochondrial intermembrane space protected against motor axonopathy and mitochondrial abnormalities in SOD1 deficient motor neurons [15].

Catalase, another component of antioxidant system, is a tetrameric enzyme that converts hydrogen peroxide to water and molecular oxygen. This enzyme is located within cells in peroxisomes and is most abundant in erythrocytes, hepatocytes, and nephrons. The *CAT* gene contains 13 exons. The common polymorphism in the promoter region of the *CAT* consists of C to T substitution at position – 262 in the 5′ region. Forsberg et al. reported that this polymorphism influences binding of transcription factor and correlates with blood CAT level [10]. Additionally, Ozkul et al. reported that the plasma level of CAT was lower in T2DM patients with neuropathy [16].

Taking into account that antioxidant enzymes are the first line defense against ROS, we aimed to evaluate (1) the plasma level of antioxidant enzymes SOD1, CAT, and GPx1 and (2) polymorphic variants *Pro197Leu* of *Gpx1*, *+35A/C* of *SOD1*, −262C/T of *CAT* in T2DM patients with and without DSPN. The association between polymorphic variants in genes coding for the scavenger enzymes like SOD1, CAT and GPx1 and the risk of diabetic neuropathy has not yet been clarified. To our best knowledge this is one of the first studies examining the relation of chosen polymorphisms in Caucasian T2DM patients with DSPN.

2. Material and methods

2.1. Study population

2.1.1. Patients

The study population consisted of 401 unrelated Caucasian individuals residing in Lodz District, Poland. Subjects were enrolled into three groups, 110 T2DM patients with DSPN, 135 T2DM patients without clinical signs and symptoms of DSPN, and 156 control subjects with normal glucose metabolism without clinical symptoms and signs of DSPN. Normoglycemia was defined as a fasting blood glucose <5.6 mmol/l and 2 h value <7.8 mmol/l. Patients were considered to have T2DM if the known diabetes was self-reported, diagnosis of T2DM was included in their medical record and if they were taking medications for management of hyperglycemia. Diagnosis of T2DM was based on the American Diabetes Association definition of diabetes [17]. Exclusion criteria included lower limb amputation, psychiatric disorders, cancer or any genetic disease, terminal illness, evidence of peripheral arterial disease, claudication symptoms, alcohol abuse, thyroid disorders, vitamin B12 or folate deficiency, spondyloarthropathy, foot edema, hepatic disease, lumbosacral pathology, toxin exposure including chemotherapeutic agents, diagnosis of neuromuscular disorders, medical or surgical intervention for peripheral nerve pathology, inability to understand or provide inform consent. The control subjects had no known diagnosis of impaired glucose metabolism and neuropathy. Patients in control group were age matched as compared to the whole group of T2DM patients. Characteristics of T2DM patients and controls are given in Table 1. All subjects were recruited from the Department of Internal Disease, Diabetology and Clinical Pharmacology between January 2010 and April 2013. The study was reviewed and approved by the Local Ethics Committee (Agreement no. RNN/210/08/KE) of the Medical University of Lodz and met the tenets of the Declaration of Helsinki. Written consent was obtained from each patient before enrolment in the study.

2.2. Diagnosis of DSPN

The diagnosis of DSPN was made based on the presence of combination of symptoms (screening questionnaire) and signs of neuropathy including decreased distal sensation and/or decreased or absent ankle reflexes after elimination of confounding factors (inclusion/exclusion criteria) [18,19]. All the sensory measurements were performed by a single clinician in a patient in supine position.

2.2.1. Symptoms of DSPN

A standardized questionnaire was completed to obtain demographic data, medical history including drugs and lifestyle factors. Symptoms of DSPN were determined from Michigan Neuropathy Screening Instrument [20].

2.2.2. Signs of DSPN

Composite score was used to assess clinical signs using modified neuropathy disability scores (NDS) comprising pinprick, vibration, temperature sensation, and Achilles reflexes [21,22].

Vibration perception was tested at the apex of the big toe with a 128 Hz graduated Rydel–Seiffer tuning fork [23,24]. The test was conducted twice on each great toe. Patients were asked to close their eyes. Before examination, the sensation of vibration was demonstrated to the patient by applying the tuning fork to the wrist. The initial sham test was performed by applying non-vibrating tuning fork in order not to mistake the sensation of pressure for vibration.

Table 1
The anthropometric data of type 2 diabetic patients with and without DSPN and control individuals. Data are expressed as mean ± SD and median and upper and lower quartiles (Q1; Q3).

	Age	BMI	HbA1c
Healthy subjects	67.22 ± 14.55 69.50 (58.00; 79.75)	27.21 ± 5.38 27.77 (24.01; 29.90)	–
T2DM patients	63.61 ± 12.97; 63 (55.00; 73.00)*	31.45 ± 5.49; 30.49 (27.44; 33.29)***	8.94 ± 2.13; 8.92 (7.35; 10.57)
T2DM with DSPN patients	68.40 ± 11.2; 69.00 (60.00; 70.00)#	30.23 ± 6.29; 29.86 (27.04; 33.69)*	8.95 ± 2.09; 8.58 (7.41; 10.17)

Mann–Whitney test $^{*}p < 0.05$ as compared to healthy subjects.
Mann–Whitney test $^{\#}p < 0.05$ as compared to T2DM patients.
t-test $^{***}p < 0.001$ as compared to healthy subjects.