Diabetic nephropathy in type 2 diabetes: MPO T-764C genotype is associated with oxidative stress

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Abstract: Background: Oxidative stress is a single mechanism relating all major pathways responsible for diabetic damage and plays an important role in diabetes development, progression and related vascular complications. To investigate the impact of oxidative stress related gene polymorphisms on development of diabetic nephropathy (DN), we tested 7 polymorphic variants that could hypothetically affect the ability of the antioxidant defense system and thus accelerate oxidative stress. Methodology: 197 Slovenian (Caucasian) type 2 diabetic (T2D) patients, age 34-83, classified into two groups according to the presence of DN, were tested for SOD2 Val16Ala (rs4880), p22 phox C242T (rs4673), CAT C-262T (rs1001179), MPO T-764C (rs2243828), GSTP1 Ile105Val (rs1695), GSTT1 and GSTM1 deletion polymorphisms using PCR, RFLP and qPCR. Oxidative stress was assessed through serum 8-hydroxy-2-deoxyguanosine (8-OHdG) level. Results were analyzed using ANOVA, Chi-square test and multivariate logistic regression. Results and Conclusions: Despite the commonly recognized link between oxidative stress and diabetes and its complications we found no association between the selected polymorphisms and DN. However, we confirmed an association between oxidative stress level and MPO T-764C genotype, which was tested in relation to DN for the first time.

Keywords: SOD2 Val16Ala • p22 phox C242T • CAT C-262T • MPO T-764C • GSTP1 Ile105Val • GSTT1 deletion • GSTM1 deletion

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1. Introduction

Diabetic nephropathy (DN) occurs in up to one third of patients after 20 years of diabetes. It is defined as a rise in urinary albumin excretion rate in the absence of other causes of renal disease. Usually, it is accompanied by retinopathy and an increase in blood pressure [1]. DN is associated with high morbidity and mortality, mainly due to cardiovascular disease (CVD) and before end-stage renal disease develops. All-cause mortality of patients with DN is 20-40 times higher in comparison to diabetic patients without DN and 2-5 times higher than with other forms of chronic kidney disease. Susceptibility to DN has a familial
basis, diabetic siblings of probands with DN have a 3-fold increase in the risk of DN. Presumably, DN is a complex, polygenic disease - genetic susceptibility is most likely determined by a large number of relatively common allelic variants, possibly interlinked and interacting with environmental influences, each individually conferring a modest increase in relative risk [1-4].

Oxidative stress is a single mechanism relating all major pathways responsible for diabetic damage. It plays an important role in diabetes development, progression and related vascular complications, including DN [5-13]. It occurs when the production of oxidants exceeds local antioxidant capacity and is derived from the two main chemical pathways: reactive oxygen species (ROS) and reactive nitrogen oxide species (RNS) [14]. Direct measurement of oxidative stress is difficult because of low oxidant serum levels and short half-life. However, it can be assessed indirectly by their oxidative byproducts, e.g. 8-OHdG, an abundant oxidative DNA product and therefore a reliable DNA damage marker [15]. There is no consensus on what its true levels are in human DNA, but a significant increase was noticed from healthy to prediabetic and finally diabetic individuals [16,17]. A marked increase was confirmed in diabetic nephropathy in comparison to diabetes without vascular complications, especially in patients with proteinuria greater than 3 g/day [18,19]. Oxidative stress can be accelerated due to an increased production of oxidants caused by hyperglycemia, and due to the a reduced ability of the antioxidant defense system.

Superoxide dismutase (SOD), probably the most important free radical scavenger, converts superoxide (O$_2^-$) into hydrogen peroxide (H$_2$O$_2$). Three SOD isoforms exist in mammals: cytosolic SOD 1 (also termed CuZnSOD), mitochondrial SOD 2 (MnSOD) and extracellular SOD 3 (EC-SOD), each derived from distinct genes but catalyzing the same reaction [20]. Catalase (CAT) has a predominant role in controlling the concentration of hydrogen peroxide [21]. Glutathione S-transferases (GSTs) inactivate secondary metabolites of ROS by catalyzing their conjugation with glutathione. GST isoforms can be classified into 7 groups: GSTA, GSTM, GSTK, GSTO, GSTP, GSTT and GSTM [22]. Some enzymes produce and utilize oxidants as a part of body's defense system, e.g. mieloperoxidase (MPO) and NADPH oxidases. MPO catalyses the conversion of hydrogen peroxide to hypochlorous acid - a cytotoxic antimicrobial agent in neutrophils and monocytes [23,24]. NADPH oxidases, a family of multi-subunit enzymes, are an important source of ROS in phagocytes and non-phagocytic cells [25].

Numerous oxidative stress-related genes are positional candidates (determined by GWAS) and candidate genes studies have confirmed the association of their polymorphisms with DN [26]. Considering these facts we analyzed the association of 7 commonly reported polymorphic variants with DN in T2D patients: SOD2 Val16Ala (rs4880), C242T polymorphism of the p22 phox gene (rs4673), encoding a subunit of NADPH oxidase; CAT C-262T (rs1001179), MPO T-764C (rs2243828), GSTT1 and GSTM1 deletion polymorphisms and GSTP1 Ile105Val (rs1695). Genotype status was compared to serum 8-OHdG, oxidative stress marker.

2. Experimental Procedures

2.1 Patients and study design
In our cross sectional study, reflecting a real diabetic population, 197 unrelated Slovenian (Caucasian) T2D patients, age 34-83, were classified into two groups according to the presence of DN: a study group of 88 patients with DN (DN+) and a control group of 109 patients without DN but T2D lasting over 10 years (DN-). Diagnosis of diabetes was made according to WHO 1999 diagnostic criteria [27]. Diabetic nephropathy was defined by increased albumin/creatinin ratio (>3 g/mol) in two out of three successive urine samples, separated by 3-month intervals; or decreased eGFR in combination with characteristic morphological changes and presence of diabetic retinopathy; both criteria in the absence of other renal disease. To avoid the confounding effect of impaired kidney function, patients with overt nephropathy were not enrolled in the study. Patients with poor glycaemic control, significant heart failure (NYHA II-IV), alcoholism, infection and other causes of renal disease were also excluded. The study was approved by the national medical ethics committee. All patients signed an informed consent for participation in the study and were interviewed in person [28].

2.2 DNA isolation and genotyping
Information on smoking, presence of CVD, family history of CVD, duration of arterial hypertension and diabetes, diabetes management and complications (retinopathy - DR, neuropathy, diabetic foot - DF), therapy and routine laboratory measurements were obtained from their medical records. DNA was extracted from peripheral blood samples using a commercial isolation kit according to manufacturer’s protocol (DNasexy Blood & Tissue Kit, Qiagen). Selected polymorphisms were tested using PCR, RFLP or real-time PCR (qPCR) with protocols in Tables 1 and 2. Genotyping was performed by two researchers (JM, DP), blinded for case or control status of the patients; duplicate samples were used. P22 phox and GST polymorphisms were tested using