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DNA REPAIR GENE OGG1 Ser326Cys POLYMORPHISM AND DNA DAMAGE IN PATIENTS WITH TYPE 2 DIABETES

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Abstract

8-hydroxydeoxyguanosine (8-OHdG) is the most frequent oxidative DNA damage. 8-oxo-deoxyguanosine DNA glycosylase 1 (OGG1) is involved in the repair of 8-OHdG. Many studies indicated that DNA repair is decreased in type 2 diabetes (T2DM). Single nucleotide polymorphisms in DNA repair genes may be linked to a decrease in DNA repair activity. The main objective of this study was to see how the OGG1 Ser326Cys gene polymorphism affected OGG1 expression and urinary excretion of 8-OHdG in T2DM patients. OGG1 expression and OGG1 genotyping in lymphocytes were detected by immuno-cytochemical staining and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism assay, respectively. Urinary 8-OHdG levels were measured by using ELISA kit in patients with T2DM. Compared with control cases, patients with T2DM had lower OGG1 immunopositivity and higher urinary 8-OHdG levels. No significant difference was found in OGG1 immunopositivity or urinary 8-OHdG levels between subjects with different OGG1 genotypes in both groups. In conclusion, The OGG1 Ser326Cys gene polymorphism has no effect on neither OGG1 expression nor urinary 8-OHdG levels. Increased urinary 8-OHdG levels despite low OGG1 immunopositivity may be derived from the action of other DNA repair enzymes.

Key words: 8-hydroxydeoxyguanosine, 8-oxo-deoxyguanosine DNA glycosylase 1, DNA repair, OGG1 polymorphism, type 2 diabetes mellitus

Introduction. Oxidative stress occurs when there is an excess reactive oxygen species (ROS) production in the body's cells and/or inadequate antioxidant capacity. One of the leading causes of type 2 diabetes mellitus (T2DM) is oxidative stress, which is exacerbated by hyperglycemia in a vicious cycle [1]. Increased ROS generation activates redox-sensitive signalling pathways, triggering pathogenic events that contribute to diabetes onset and progression. Hyperglycemia aggravates oxidative stress after the development of diabetes because of the increased synthesis of advanced glycation end-products. Oxidative DNA damage is an underlying pathogenic mechanism in the development of T2DM and its long-term complications [2].

Recent evidence indicates that there is high oxidative DNA damage and reduced DNA repair ability in cases with T2DM [3]. However, little is known about whether decreased DNA repair ability is a result of decreased expression of the genes encoding the DNA repair proteins or single nucleotide polymorphisms (SNPs). DNA repair genes have several single nucleotide polymorphisms (SNPs) that can affect the function of their encoded proteins. 8-hydroxydeoxyguanosine (8-OHdG) is the most frequent oxidative DNA lesion. 8-oxo-deoxyguanosine DNA glycosylase 1 (OGG1) excises 8-OHdG adducts. Serum and urinary 8-OHdG is a biomarker for oxidative DNA damage. Decreased function of OGG1 causes an increase in 8-OHdG lesions in cells. The Ser326Cys polymorphism has been suggested to decrease DNA repair activity [4]. Gene-ROS interaction may increase the risk for T2DM among individuals with OGG1 Ser326Cys polymorphism. In the present study, OGG1 expression, OGG1 genotyping, and urinary 8-OHdG concentration were examined in subjects with T2DM to evaluate the causal link between OGG1 expression and urinary excretion of 8-OHdG and to assess the influence of the OGG1 Ser326Cys polymorphism.

Patients, materials and methods. *Patients.* The study included 250 individuals with T2DM and 200 control cases recruited from the Department of Internal Medicine. The characteristic data of the study groups are given in Table 1. A total of 196 subjects were taking oral antidiabetic agents, 20 subjects were treated with insulin, and 34 were given both of these medication treatments. Thirty-eight individuals in the patient group had at least one complication such as neuropathy, retinopathy, kidney disease, or heart disease. The control group consisted of 200 non-diabetic cases without a family history of diabetes who were age- and sex-matched. They were recruited among individuals having blood glucose levels lower than 140 mg/dl at the 2nd hour of oral glucose tolerance testing. The subjects in this study were non-smokers. The participants who had been taking oral antioxidant or iron supplementation in the prior three months were excluded. Infectious disease, inflammatory disease, autoimmune disorders, or any malignant disease were exclusion criteria.

After a 12-hour overnight fast, EDTA-containing blood samples and spot urine samples were collected from study participants. Blood samples were pro-

Demographic and laboratory data of the study groups

	Control	T2DM	p
Age (year)	53 (20–83)	55 (18–84)	0.090
Sex			
Men	79	104	
Women	121	146	
BMI (kg/m^2)	30 (20–56)	31 (17–48)	0.064
FBG (mg/dl)	96 (68–120)	136 (71–457)	**0.000**
HbA1c (%)	5.60 (4.60–6.00)	7.20 (5.00–14.20)	**0.000**
(mmol/mol)	39 (27–45)	55 (31–132)	
Insulin (µl/ml)	8.21 (3.38–35.81)	10.34 (0.20–91.47)	**0.015***
HOMA-IR	1.98 (0.10–6.00)	3.47 (0.13–27.35)	**0.000***
C-peptide (ng/ml)	1.76 (0.53–5.99)	1.86 (0.05–4.79)	0.394
CRP (mg/dl)	0.33 (0.02–12.43)	0.68 (0.01–30.69)	**0.003**
8-OHdG (ng/mg creatinine)	5.72 (0.69–34.22)	10.04 (0.23–77.25)	**0.000**

FBG: Fasting blood glucose

HOMA-IR: Homeostasis model assessment of insulin resistance

CRP: C-reactive protein

*The patients treated with insulin were excluded in the statistical analysis

cessed immediately, and urine samples were stored at $-80\,^\circ$C until they were used to assess the 8-OHdG level.

OGG1 expression. Immunocytochemical staining was used to evaluate the expression of OGG1 protein in peripheral blood leukocytes (PBLs), as described previously [5]. In a one hour incubation at room temperature, OGG1 protein was detected on formalin-fixed, PBS-diluted cell smears at 1/100 with the rabbit polyclonal OGG1 antibody (NOVUS, Anti-OGG1 antibody NB100-105). The specimens were treated with biotin-labelled secondary antibody for 10 min followed by the addition of avidin-biotin-peroxidase complex (Invitrogen Histostain-Plus Bulk Kit, Cat No 85-9043) for 8 min at room temperature. The samples were visualized with amine nickel sulphate-enhanced 3,3′-diaminobenzidine (DAB) method. After that, the cells were counterstained with methyl green and rinsed with distilled water. Then the samples were evaluated under light microscopy and the development of brown particles indicated positive staining. For negative control reactions, PBS was used instead of the primary antibody. Under ×20 magnification, positive staining was quantitatively evaluated in randomly chosen 12 fields.

Genotyping of OGG1 Codon 326. The genotyping of OGG1 codon 326 was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. The genomic DNA was isolated from peripheral blood lymphocytes pellets using a Nucleo-Spin DNA purification kit
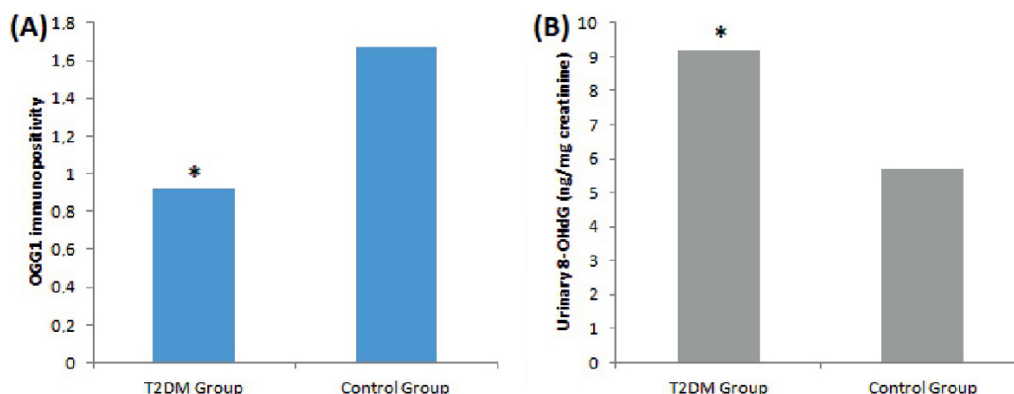
Fig. 1. OGG1 immunopositivity (A) and urinary 8-OHdG levels (B) in the T2DM and control groups ($^*p < 0.001$ vs. Control group)

(Macherey–Nagel GmbH, Duren, Germany). The OGG1 Ser326Cys polymorphism was genotyped using PCR-RFLP with primers HOGG1F: 5′-GGAAGGTG CTTGGGGAAT-3′ and HOGG1R: 5′-ACTGTCACTAGTCTCACCAG-3′, as described previously [6]. PCR products were confirmed by real-time PCR.

Urinary 8-OHdG level. Urinary 8-OHdG levels were determined by median values of the enzyme-linked immunosorbent assay (ELISA), using human ELISA kits (Northwest Life Science Specialties, Vancouver, WA) with the test procedure suggested by the manufacturer. Findings were normalized with urinary creatinine concentration.

Statistical analysis. IBM SPSS Statistics 20 was used to examine the data. Continuous variables were described as median (min-max). Differences among the groups in terms of genotype and allele distribution were determined using the Chi-square test and the genotype frequencies for each polymorphism of OGG1 were tested for deviation from the Hardy–Weinberg equilibrium. The results of the immunocytochemical staining of OGG1 were evaluated using the GraphPad InStat tool, and comparisons between two groups were made with the Mann–Whitney U test. The correlation between the variables was investigated using the Spearman correlation coefficient. Significance was accepted at $p < 0.05$.

Results. Insulin resistance was determined with HOMA-IR (Homeostasis model assessment-Insulin resistance) calculated by insulin (mIU/L) × glucose (mmol/L)/22.5. The T2DM group had higher fasting glucose, insulin, CRP, HbA1c levels, and HOMA-IR levels in their blood than the control group (Table 1). Urinary 8-OHdG levels were higher ($p < 0.001$), OGG1 immunopositivity was lower ($p < 0.001$) in T2DM group than those in the control group (Fig. 1). No correlation was determined between urinary 8-OHdG level and fasting glucose, insulin, c-peptide, CRP, HbA1c, HOMA-IR, and body mass index (BMI). None of these variables were correlated with OGG1 immunopositivity. No correlation was found between OGG1 positivity and urinary 8-OHdG levels. No significant

The distribution of OGG1 Ser326Cys genotypes in the T2DM and control groups

	T2DM ($n = 250$)	Control ($n = 200$)	OR (95% Cl)	p
Ser/Ser	140 (56%)	132 (66%)	Ref.	
Ser/Cys	90 (36%)	50 (25%)	0.60 (0.32–1.10)	0.11
Cys/Cys	20 (8%)	18 (9%)	0.97 (0.36–2.60)	0.87
G (Ser) allele frequency	0.74	0.78	Ref.	
C (Cys) allele frequency	0.26	0.22	0.80 (0.40–1.61)	0.62

difference was found between treated and untreated patients for urinary 8-OHdG and OGG1 immunopositivity. The OGG1 Ser326Cys genotypes distribution in subjects was consistent with the Hardy–Weinberg equilibrium and was demonstrated in Table 2. The OGG1 Ser326Cys genotypes distribution ($p > 0.05$) and allele ($p > 0.05$) frequencies between patients and control subjects ($p > 0.05$) did not reach statistical significance. The study did not reveal any significant differences between individuals carrying different OGG1 genotypes (Ser326Ser, Ser326Cys and Cys326Cys) for OGG1 immunopositivity and urinary 8-OHdG levels in T2DM and control subjects.

Discussion. In the present study, for the first time, lower OGG1 immunopositivity in PBLs smear preparations obtained from patients with T2DM was detected. This finding clearly indicates that repair of oxidative DNA damage is diminished in patients with T2DM. As far as we know, no research examining the link between OGG1 expression (at neither mRNA nor protein level) and urinary 8-OHdG excretion in T2DM patients was reported. The 8-OHdG level in the urine reflects its rate of production and repair. If its repair is defective, 8-OHdG may not increase in the urine although a high level of oxidative DNA damage is present at the tissue level. However, both OGG1 expression and urinary 8-OHdG level were not examined in the same study to clarify the possible causal relationship. In a previous study urinary 8-OHdG excretion [2] was found to be higher in patients with T2DM. They are different studies and their findings are far from the explanation whether these increases are related to OGG1 expression level. In a recent in vitro study, metformin was shown to decrease endothelial ROS production by increasing OGG1 protein expression [7]. Although some patients were on metformin treatment in our study group, no significant difference was found between treated and untreated patients with T2DM for neither urinary 8-OHdG level nor OGG1 immunopositivity.

If OGG1 expression is decreased, lower urinary 8-OHdG excretion is expected due to the reduced repair. Surprisingly, we found higher urine 8-OHdG levels despite lower OGG1 immunopositivity in the patients with T2DM. This finding brings to mind that 8-OHdG damage may also be repaired by an enzyme other

than OGG1. hMTH1 (human mutT homologue 1) gene encodes an oxidized purine nucleoside triphosphatase that removes 8-OHdG adducts [8]. The contributory repair activity of hMTH1 towards 8-OHdG may be the answer to the question why urinary 8-OHdG levels were found to be higher despite lower OGG1 immunopositivity.

Subjects with the OGG1 Cys326/Cys326 genotype were found to have a higher expression level of OGG1 mRNA than wild-type allele carriers [9]. There are many studies indicating that OGG1 Ser326Cys polymorphism is linked with the diminished repair activity of OGG1 [4, 10]. Although opposite data is also available [11] a positive association between OGG1 Ser326Cys polymorphism and insulin sensitivity, diabetes or diabetic complications are reported by several groups [12–14]. In the previous studies, OGG1 Ser326Cys polymorphism was examined as a risk factor for the development of diabetes. However, the data about the effect of OGG1 Ser326Cys polymorphism on the repair of the 8-OHdG lesion is limited. WU et al. [13] determined that OGG1 mRNA is lower and plasma 8-OHdG level is higher in T2DM patients carrying the Cys/Cys genotype; OGG1 Ser326Cys polymorphism shows a correlation with coronary artery lesions in T2DM patients. In our study, the OGG1 immunopositivity and the levels of urinary 8-OHdG were not found to be influenced by OGG1 Ser326Cys polymorphism.

The causal relationship between OGG1 Ser326Cys polymorphism and urinary 8-OHdG level was assessed in a few studies from different research areas and their findings are inconsistent. Urinary 8-OHdG level has been reported to be higher in Chinese and Vietnamese individuals carrying OGG1 Cys326Cys genotype compared with individuals carrying Ser326Cys and Ser326Ser genotypes [15, 16]. However, in a study carried on styrene-exposed workers no statistically significant association was found between urinary 8-OHdG levels and OGG1 Ser326Cys polymorphism [17].

Conclusion. The present study shows that OGG1 Ser326Cys gene polymorphism has no effect on OGG1 expression and urinary 8-OHdG levels. As a limitation of this study, OGG1 enzyme activity was not measured and it is not clear whether decreased OGG1 immunopositivity is associated with decreased repair activity. Further studies at the cell level are required to clarify this issue. Determination of OGG1 immunopositivity, OGG1 enzyme activity and amount of 8-OHdG adducts on DNA in leukocytes may be more informative. In addition, measurement of repair activity of hMTH1 in such an experimental design is also helpful to clarify whether decreased OGG1 immuno-positivity is associated with decreased repair activity. If a causal relationship is determined between DNA repair gene polymorphism and oxidative DNA damage biomarkers, a new perspective may open in prevention of diabetes. Development of diabetes may be slowed or prevented by oxidative stress-reducing approaches in individuals with these DNA repair gene polymorphisms.

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