

Urinary 8-hydroxydeoxyguanosine levels in diabetic retinopathy patients

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PURPOSE. *Involvement of oxidative stress in the pathogenesis of diabetic microvascular complications has been proposed. Recently, 8-hydroxy-2'-deoxyguanosine (8-OHdG) has been reported to serve as a new sensitive biomarker of the oxidative DNA damage in vivo. This study was undertaken to investigate whether the urinary levels of 8-OHdG are altered in patients with type 2 diabetes. The authors also attempted to analyze the relationship between 8-OHdG levels and other clinical parameters of patients with diabetes, especially the relationship between oxidative DNA damage and the severity of the retinal lesions in patients with diabetic retinopathy.*

METHODS. *The authors studied 60 patients with type 2 diabetes and compared them with 35 nondiabetic control subjects. Urinary 8-OHdG concentrations were assayed using competitive enzyme-linked immunosorbent assay.*

RESULTS. *The patients with type 2 diabetes had significantly higher concentrations of 8-OHdG in their urine than the control subjects (19.6±6.7 vs 11.9±4.9 ng/mgCr; p<0.05). The authors could not find any correlation between urinary 8-OHdG levels and age, duration of diabetes, or serum lipids. However, HbA1c values were significantly correlated with 8-OHdG values. Among the patients with diabetes, those with proliferative retinopathy had significantly higher 8-OHdG levels than those with nonproliferative retinopathy or without retinopathy.*

CONCLUSIONS. *The authors' findings show that measuring urinary 8-OHdG is a novel convenient method for evaluating oxidative DNA damage in patients with diabetes, and it is also suggested that 8-OHdG could be a sensitive biomarker and may be helpful for the early diagnosis and treatment of patients with diabetic retinopathy. (Eur J Ophthalmol 2008; 18: 94-8)*

KEY WORDS. *8-hydroxy-2'-deoxyguanosine (8-OHdG), Diabetic retinopathy*

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INTRODUCTION

The number of adults with clinical diagnosis of diabetes has been increasing dramatically worldwide. Hyperglycemia resulting from uncontrolled glucose regulation is widely recognized as the causal link between diabetes and diabetic complications. Four main hypotheses have been presented to describe how hyperglycemia can cause all of these diabetic complications (1): increased polyol pathway flux, increased advanced glycation end-product (AGE) formation, increased protein kinase C iso-

form expression, and increased hexosamine pathway flux. Until recently, there was no unifying hypothesis linking these four mechanisms. It has been shown that hyperglycemia induced overproduction of superoxide is the trigger that drives each of these pathways (2). Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus (I and II). Oxidative stress appears to be the pathogenic factor in underlying diabetic complications. Free radicals are formed disproportionately in diabetes by glucose oxida-

tion, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Consequently, the free radicals thus generated promote the development of complications of diabetes mellitus (3).

Various markers of oxidative damage have been identified (4). In the past, the most popular markers were designed for lipid peroxidation, such as malondialdehyde (MDA), oxidized LDL, MDA-modified LDL, autoantibodies against oxidized LDL and MDA-modified LDL, F₂-isoprostane, and conjugated diene. The detection of a new carbonyl group, dityrosine and oxidized histidine, has been measured to indicate protein oxidation. Now it is well known that the study of oxidative DNA damage is clinically important (4). Among the many types of modifications induced by oxidative stress, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of the abundant products of oxidative DNA damage (5). Numerous studies have shown that oxidative DNA damage links pathogenically to a variety of aging-associated degenerative diseases such as cancer, coronary heart disease, and diabetes. Only in recent years has 8-OHdG emerged as a new sensitive biomarker of the in vivo oxidative DNA damage in diabetes (6, 7). Urinary 8-OHdG, in particular, has been measured most frequently to indicate the extent of oxidative damage because it is noninvasive and technically less involved. Quantification of urinary 8-OHdG, a specific DNA repair product in the urine, can be made with a simple competitive enzyme-linked immunosorbent assay (ELISA) (8).

Diabetic retinopathy is a common microvascular complication that increases the risk of blindness and can severely affect quality of life for patients with diabetes. Early diagnosis and treatment of diabetic retinopathy is critical. This study was undertaken to investigate whether the urinary level of 8-OHdG is altered in patients with type 2 diabetes, especially in patients with diabetic retinopathy. We also attempted to analyze the relationship between 8-OHdG levels and other clinical parameters of patients with diabetes.

MATERIALS AND METHODS

Patients and controls

We studied 60 patients with type 2 diabetes and 35 nondiabetic age-matched control subjects at the Shandong University Qilu Hospital, Shandong, China. The diagnosis of type 2 diabetes was based on clinical characteristics,

including no episode of ketoacidosis, a diagnosis of diabetes after 25 years old, and treatment by diet or oral hypoglycemic agents or a fasting serum C-peptide value >0.30 nmol/L in patients using insulin. Funduscopic examination was performed using an ophthalmoscope, and the patients were classified into groups according to retinopathy (no signs of diabetic retinopathy, nonproliferative diabetic retinopathy [NPDR], or proliferative diabetic retinopathy [PDR]). Control subjects showed normal glucose tolerance and had no family history of diabetes. None of our subjects had any acute illness or chronic condition at the time of the study. None of the subjects were taking any medication that would have affected the assay of the 8-OHdG. All patients were normotensive and nonsmokers. Informed consent was obtained prior to enrollment.

Sample collection

Early morning voiding urine samples were obtained from each subject. The samples were centrifuged and the supernatants were stored at -20 °C until analysis. Peripheral blood was collected at fasting state, and serum cholesterol and triglyceride levels were measured via an autoanalyzer with enzymatic technique. HbA_{1c} level was measured by high-performance liquid chromatography (HPLC).

Determination of urinary markers of oxidative stress

The urinary concentrations of 8-OHdG were determined in duplicate using competitive ELISA kits (8-OHdG Check, Institute for the Control of Aging, Shizuoka, Japan). These urinary markers were expressed relative to urinary Cr concentration, which was measured by picrate method. All the analyses were performed in duplicate, and the examiners were blinded to the clinical and laboratory results. The determination of urinary 8-OHdG level was performed mainly as follows.

The 8-OHdG monoclonal antibody and the sample or standard are added to the microtiter plate which has been precoated with 8-OHdG. The 8-OHdG monoclonal antibody reacts competitively with the 8-OHdG bound on the plate and the 8-OHdG in samples solution. Therefore higher concentrations of 8-OHdG in the sample solution lead to a reduced binding of the antibody to the 8-OHdG on the plate.

The antibodies which are bound to the 8-OHdG in the sample are washed away from the antibodies that have bound to the 8-OHdG coated on the plate.

An enzyme-labeled secondary antibody, which is added to the plate, binds to the monoclonal antibody which is bound to the 8-OHdG coated on the plate.

Unbound enzyme-labeled secondary antibody is removed by a wash step.

Addition of a chromatic substrate results in the development of color in proportion to the amount of antibody bound to the plate.

The color reaction is terminated and the absorbance is measured.

Statistical analysis

Student *t*-test was used to compare the level of 8-OHdG between diabetic patients and control subjects. One-way analysis of variance (one-way ANOVA) was used to compare HbA1c and 8-OHdG levels between subgroups of different severities of complications of patients with diabetes. Correlation between urinary 8-OHdG and other independent parameters was performed using Pearson correlation. Data in the text were given as mean±SD. Differences of $p < 0.05$ were considered statistically significant.

RESULTS

The biochemical, clinical, and demographic characteristics of patients with diabetes and control subjects are listed in Table I. Urinary concentrations of 8-OHdG were significantly increased in patients with diabetes compared with their controls (19.6±6.7 vs 11.9±4.9 ng/mgCr,

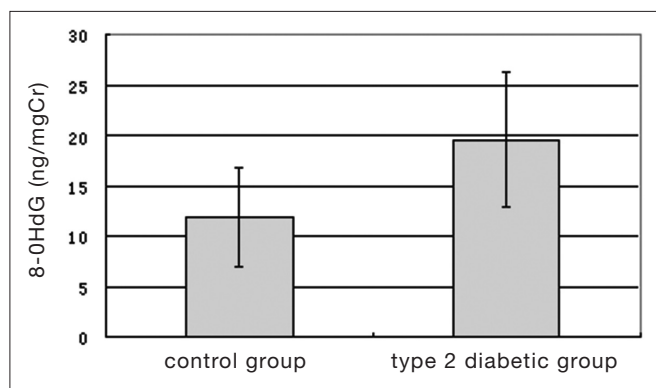


Fig. 1 - Urinary concentrations of 8-OHdG were significantly higher in the patients with type 2 diabetes than in the healthy controls (19.6±6.7 vs 11.9±4.9 ng/mgCr, $p < 0.05$ by Student *t* test.

$p < 0.05$) (Fig. 1). In the diabetic group, when we subdivided the patients into three groups according to the presence and severity of retinopathy (no retinopathy, NPDR, and PDR), subjects with PDR (26.2±4.5 ng/mgCr) had significantly higher 8-OHdG levels than subjects with NPDR (18.7±3.6 ng/mgCr) or subjects without retinopathy (12.6±2.6 ng/mgCr) (Fig. 2). We could not find any correlation between urinary 8-OHdG level and age, duration of diabetes, serum total cholesterol level, or triglyceride level (data not shown). For the diabetic group, HbA1c values were significantly correlated with 8-OHdG ($r = 0.53$, $p < 0.05$) (Fig. 3).

DISCUSSION

Evidence has been accumulated indicating that generation of reactive oxygen species (ROS) and oxidative stress

TABLE I - BIOCHEMICAL, CLINICAL, AND DEMOGRAPHIC CHARACTERISTICS OF DIABETIC PATIENTS AND CONTROL SUBJECT (mean ± SD)

Group	n (male/female)	Age (years)	BMI (kg/m ²)	Duration (years)	HbA1c (%)	Triglyceride (mmol/L)	Cholesterol (mmol/L)	8-OHdG (ng/mgCr)
Control	35 (20/15)	60 ± 6	22.9 ± 0.6	-	-	1.3 ± 0.8	4.9 ± 0.8	11.9 ± 4.9
Diabetic patients	60 (32/28)	62 ± 10	23.2 ± 0.5	11 ± 8	9.1 ± 1.7	1.5 ± 0.9	5.0 ± 0.7	19.6 ± 6.7°
no retinopathy	18	61 ± 11	-	7 ± 7	8.1 ± 1.1	1.6 ± 1.2	5.0 ± 1.4	12.6 ± 2.6*
NPDR	20	64 ± 9	-	10 ± 9	9.3 ± 1.4*	1.4 ± 0.9	4.9 ± 1.0	18.7 ± 3.6*
PDR	22	64 ± 12	-	11 ± 8	9.7 ± 2.0*	1.5 ± 0.8	5.1 ± 0.9	26.2 ± 4.5

$p < 0.05$ * Versus no retinopathy group; ° Versus control groups; * Versus PDR group

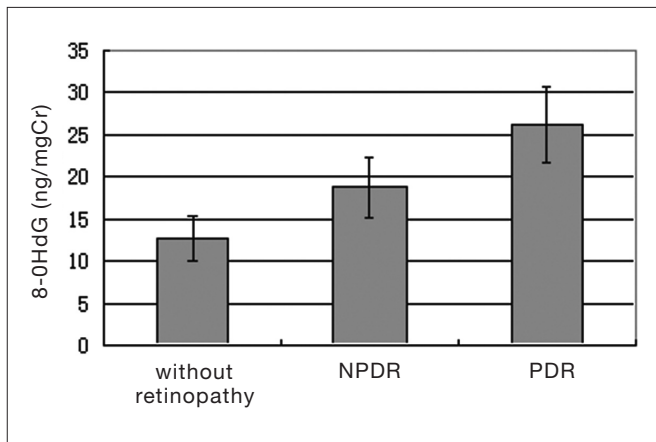


Fig. 2 - Urinary 8-OHdG was significantly higher in patients with proliferative diabetic retinopathy (26.2 ± 4.5 ng/mgCr) than those with nonproliferative diabetic retinopathy (NPDR) (18.7 ± 3.6 ng/mgCr) or subjects without retinopathy (12.6 ± 2.6 ng/mgCr). There was a significant difference between patients with NPDR and those without retinopathy ($p < 0.05$ by one-way analysis of variance).

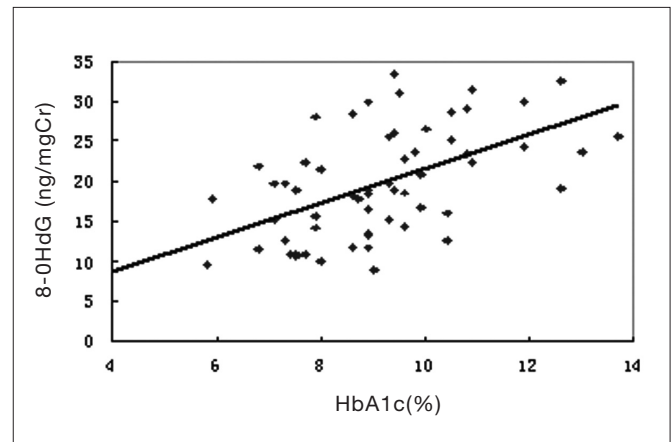


Fig. 3 - Relationship between hemoglobin A1c (HbA1c) and urinary 8-OHdG concentration in patients with type 2 diabetes. HbA1c values were positively correlated with 8-OHdG ($r = 0.53$, $p < 0.05$).

play an important role in the pathogenesis of diabetic complications (9, 10). Diabetic retinopathy, a debilitating microvascular complication of diabetes, and many diabetes-induced metabolic abnormalities are also implicated in its development and appear to be influenced by elevated oxidative stress (11). 8-OHdG is one of the major forms of oxidative DNA damage (12). Damaged DNA is usually extracted by repair enzymes and the resulting free water-soluble 8-OHdG is excreted without further metabolism into the urine. Currently, 8-OHdG is one of the most popular markers for oxidative DNA damage and oxidative stress in vivo (13).

In the present study, we found significantly increased urinary concentrations of 8-OHdG in patients with type 2 diabetes compared with the corresponding controls. This result is in agreement with an earlier report by Dandona et al (14) who observed a higher concentration of 8-OH-dG in mononuclear cells of patients with type 1 and type 2 diabetes compared with control subjects. Of interest in our study is that urinary 8-OHdG levels are increased significantly in the patients with PDR and NPDR, especially in the PDR. Although little is known about the participation of oxidative stress in retinopathy, Grattagliano et al (15) demonstrated an increase in MDA and carbonyl proteins and a decrease in vitamin E and sulfhydryl proteins in subretinal fluid of patients with diabetes, especially in those patients with PDR. Studies

(16, 17) have found that in diabetes, oxidative metabolites can stimulate cell proliferation by increasing the amounts of different growth factors such as vascular endothelial growth factor. The authors speculated that in hyperglycemia, the relative increase of oxidative metabolites contributes both to the damage of retinal vessels and to a more pronounced proliferative activity in diabetic retinopathy. They also found that strict blood glucose control leads to reduced oxidative tissue damage both systemically and locally in the eye, and reduces proliferative activity. Of note, glycemic control (expressed by HbA1c values) was significantly correlated with urinary 8-OHdG. This result suggests the connection between glycemic status and oxidative DNA damage in patients with diabetes. Studies of larger scale are needed to confirm the results of the present study. In our study, increased urinary 8-OHdG levels in patients with diabetes were associated with advanced complications, but not with duration of diabetes. Currently, the reason for such a discrepancy is not clear, but it might be because of the late diagnosis of diabetes in some subjects. Thus, the known duration of diabetes may not reflect the real duration of disease in some patients (18).

In conclusion, urinary 8-OHdG concentration in patients with type 2 diabetes was significantly increased compared with that of control subjects, and 8-OHdG con-

centration was even higher in the patients with advanced diabetic retinopathy. Hyperglycemia is likely to have contributed to the increase in 8-OHdG levels. These results allow us to conclude that patients with diabetes show greater oxidative damage to DNA, which might play a role in the pathogenesis of diabetic complications. Therefore, it is suggested that 8-OHdG could be a sensitive biomarker and may be helpful for the early diagnosis and treatment of patients with diabetic complications. Moreover, oxidative injury is likely to be dependent on the balance between free radical generation and antioxidant defense of tissue. Further studies should investigate the role of antioxidant treatment in patients with type 2 diabetes as a potential therapy for the prevention of diabetic retinopathy.

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