Serum 8-Hydroxy-Guanine Levels Are Increased in Diabetic Patients

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OBJECTIVE — The production of reactive oxygen species is increased in diabetic patients, especially in those with poor glycemic control. We have investigated oxidative damage in type 2 diabetic patients using serum 8-hydroxyguanine (8-OHG) as a biomarker.

RESEARCH DESIGN AND METHODS — We studied 41 type 2 diabetic patients and compared them with 33 nondiabetic control subjects. Serum 8-OHG concentration was assayed using high-pressure liquid chromatography.

RESULTS — The type 2 diabetic patients had significantly higher concentrations of 8-OHG in their serum than the control subjects (5.03 ± 0.69 vs. 0.96 ± 0.15 pmol/ml; $P < 0.01$). There was no association between the levels of 8-OHG and HbA_{1c}. We also could not find any correlation between serum 8-OHG levels and age, duration of diabetes, serum lipids, or creatinine or albumin excretion rate. Creatinine clearance showed marginal correlation with serum 8-OHG levels ($P = 0.06$). Among the diabetic patients, those with proliferative retinopathy had significantly higher 8-OHG levels than those with nonproliferative retinopathy or without retinopathy. Likewise, the serum 8-OHG levels in patients who had advanced nephropathy (azotemia) were higher than in patients with normoalbuminuria, microalbuminuria, or overt proteinuria.

CONCLUSIONS — Our findings show that measuring serum 8-OHG is a novel convenient method for evaluating oxidative DNA damage. Diabetic patients, especially those with advanced microvascular complications, had significantly higher serum 8-OHG levels; this suggests that such changes may contribute to the development of microvascular complications of diabetes.

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Considerable evidence has been accumulated to suggest that the production of reactive oxygen species (ROS) is increased in diabetic patients, especially in those with poor glycemic control (1–3). Excessive ROS can accelerate oxidative damage to macromolecules, including lipids and proteins, as well as to DNA. An increased production of malondialdehyde (MDA), a marker of lipid peroxidation, has been demonstrated in the erythrocyte membranes of diabetic patients, along with a depressed erythrocyte content of reduced glutathione (4). Moreover, there is a significant relationship between markers of lipid peroxidation and metabolic control in both type 1 and type 2 diabetic patients (5). Although there are fewer data regarding the oxidation of protein, a recent study by Suzuki et al. (6) demonstrated local oxidative stress and carbonyl modification of proteins in diabetic glomerulopathy.

8-Hydroxydeoxyguanosine (8-OH-dG), an ROS-induced modification of a purine residue in DNA, is a sensitive index of oxidative DNA damage (7). 8-OH-dG in plasma increases with age (8), with cigarette smoking (9), and during tumorigenesis (10). The urinary level of this molecule is now considered a biomarker of the total systemic oxidative stress in vivo. Dandona et al. (11) demonstrated that type 1 and type 2 diabetic patients had a significantly higher concentration of 8-OH-dG in their mononuclear cells than the respective control subjects. Urinary excretion of 8-OH-dG has also been shown to be higher in both type 1 and type 2 diabetic patients compared with nondiabetic subjects (3,12). Different methods have been used for the analysis of 8-OH-dG, but high-pressure liquid chromatography (HPLC) with electrochemical detection (ECD) is highly sensitive and is frequently used for this purpose (13,14). However, during the preparation of a sample for HPLC, there is a risk of auto-oxidation of deoxyguanosine (dG), which would result in a false high background as well as low sensitivity (15). In contrast, the HPLC-ECD measurement of serum level of 8-hydroxy-guanine (8-OHG), a free base of 8-OH-dG, is not affected by the work-up procedures and is well correlated with the oxidative DNA damage of the tissues (14). This study was undertaken to investigate whether the serum level of 8-OHG is altered in type 2 diabetic patients. We also attempted to analyze the relationship between 8-OHG levels and other clinical parameters of diabetic patients.

RESEARCH DESIGN AND METHODS

— We studied 41 type 2 diabetic patients and 33 nondiabetic age-matched control subjects at the Seoul National University Hospital, Seoul, Korea (Table 1). The diagnosis of type 2 diabetes was based on clinical characteristics, including no episode of ketoacidosis, a di-

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Abbreviations: AER, albumin excretion rate; dG, deoxyguanosine; ECD, electrochemical detection; HPLC, high-pressure liquid chromatography; MDA, malondialdehyde; NPDR, nonproliferative diabetic retinopathy; 8-OH-dG, 8-hydroxydeoxyguanosine; 8-OHG, 8-hydroxyguanine; PDR, proliferative diabetic retinopathy; ROS, reactive oxygen species.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Biochemical, clinical, and demographic characteristics of diabetic patients and control subjects*

	Diabetic patients	Control subjects
n (male/female)	41 (16/25)	33 (17/16)
Age (years)	57.2 ± 2.1	51.7 ± 1.4
BMI (kg/m^2)	23.2 ± 0.6	23.7 ± 0.5
Duration of diabetes (years)	11.8 ± 1.1	—
HbA_{1c}(%)	9.1 ± 0.3	—
Glucose (mmol/l)	8.8 ± 0.4*	4.9 ± 0.1
Cholesterol (mmol/l)	4.99 ± 0.19	4.91 ± 0.16
Triglyceride (mmol/l)	1.73 ± 0.14	1.66 ± 0.18
HDL-cholesterol (mmol/l)	1.11 ± 0.06	1.34 ± 0.09
Creatinine (μmol/l)	84.0 ± 4.6†	68.4 ± 2.1

Data are means ± SE. *$P < 0.001$ vs. control subjects. †$P < 0.01$ vs. control subjects.

agnosis of diabetes after 25 years of age, and treatment by diet or oral hypoglycemic agents or a fasting serum C-peptide value >0.30 nmol/l in patients using insulin. Fundoscopic 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], and proliferative diabetic retinopathy [PDR]). Patients were instructed to collect 24-h urine samples for the determination of the albumin excretion rate (AER). Normoalbuminuria was considered to be present when AER was <20 μg/min. Microalbuminuria was defined as AER 20–200 μg/min, and overt proteinuria was defined as AER >200 μg/min with serum creatinine concentration ≤106 μmol/l. We classified patients as having azotemia when serum creatinine concentration was >106 μmol/l. According to these criteria, 61% (25 of 41) of patients had retinopathy (12 NPDR and 13 PDR), and 73% (30 of 41) had nephropathy (9 microalbuminuria, 11 overt proteinuria, and 10 azotemia). Control subjects showed normal glucose tolerance and had no family history of diabetes. There was no difference in either demographic or biochemical parameters other than glucose tolerance and creatinine levels between control subjects and diabetic patients. None of the subjects were taking any medication that would have affected the assay of the 8-OHG. All subjects gave written informed consent, and the ethics committees of the Seoul National University Hospital approved this study.

Peripheral blood was collected at fasting state, and serum glucose, creatinine, cholesterol, triglyceride, and HDL-cholesterol levels were measured via an autoanalyzer with enzymatic technique. HbA_{1c} level was measured by affinity chromatography. Serum C-peptide was measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA).

Determination of the serum 8-OHG level was performed as previously described (16). Briefly, after removing 8-OH-dG from the serum, the sample was applied to an immunoaffinity column of monoclonal antibody to 8-OHG (provided by Dr. Bruce Ames, University of California, Berkeley, CA). The amount of 8-OHG in the elutant was measured by an HPLC-ECD.

All data presented are expressed as mean ± SE. Student's *t* test was used to compare the level of 8-OHG between diabetic patients and control subjects. Analysis of variance with Duncan's multiple range test was used for the comparison among subgroups of different severities of complications. Correlation between serum 8-OHG and other independent parameters was performed using Pearson's correlation.

RESULTS — The serum 8-OHG concentrations in diabetic patients were significantly higher than the concentrations in control subjects (5.03 ± 0.69 vs. 0.96 ± 0.15 pmol/ml; $P < 0.01$, Fig. 1). In the diabetic group, no significant correlation was observed between serum 8-OHG and HbA_{1c} levels. In addition, we could not find any correlation between serum 8-OHG level and age, duration of diabetes, serum total cholesterol, HDL cholesterol level, triglyceride level, creatinine level, or AER. Creatinine clearance showed marginal correlation with serum 8-OHG levels ($P = 0.06$, Table 2). When we subdivided the patients into three groups according to the presence and severity of retinopathy (no retinopathy, NPDR, and PDR), subjects with PDR (8.27 ± 0.31 pmol/ml) had significantly higher 8-OHG levels than subjects with NPDR (4.92 ± 0.34 pmol/ml), or subjects without retinopathy (3.38 ± 0.22

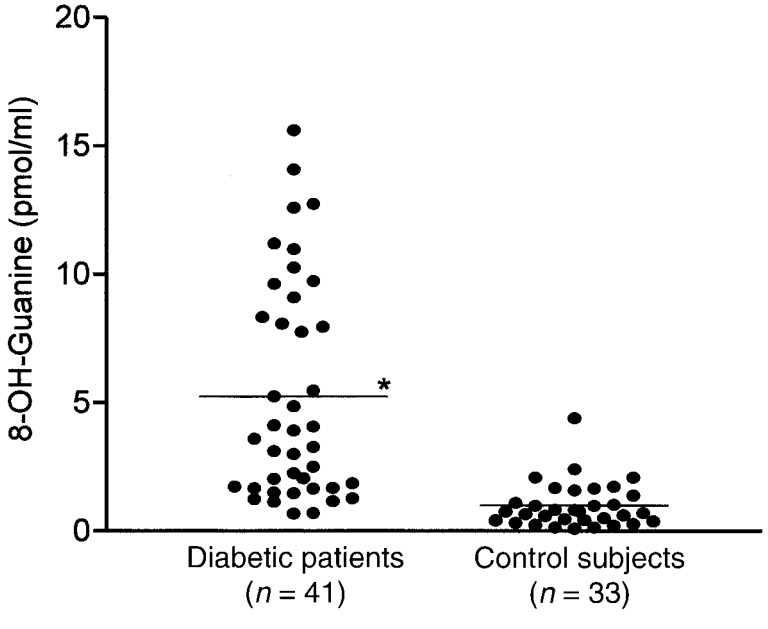

Figure 1—*Serum 8-OHG levels in type 2 diabetic patients and control subjects. *P < 0.01 vs. control subjects. The line represents mean value.*

Table 2—*Correlation of serum 8-hydroxyguanine levels with other parameters in the diabetic group* (n = 33)

	Correlation coefficient	*P*
Age	0.22	0.16
BMI	0.03	0.29
Duration of diabetes	0.10	0.21
HbA_{1c}	0.12	0.23
Cholesterol	0.07	0.45
Triglyceride	–0.14	0.25
HDL cholesterol	0.22	0.12
Creatinine	0.30	0.10
Creatinine clearance	–0.34	0.06
AER	0.32	0.14

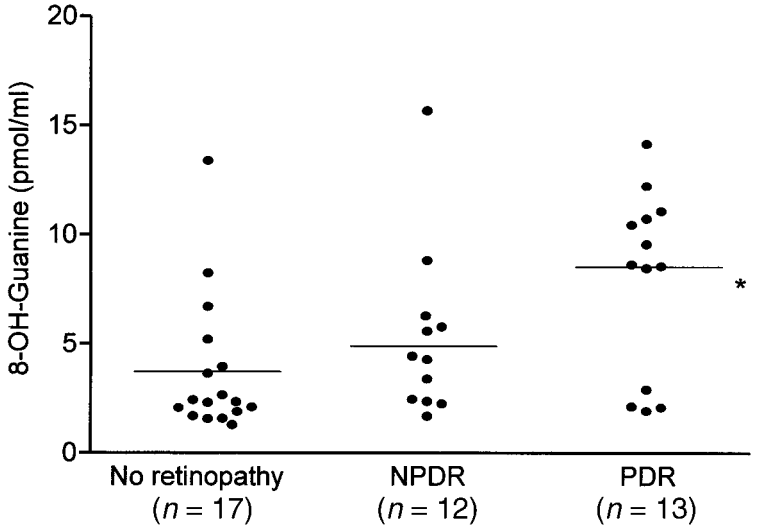

Figure 2—*Serum 8-OHG levels in type 2 diabetic patients according to diabetic retinopathy group. *P < 0.01 vs. no retinopathy and NPDR. The line represents mean value.*

pmol/ml) (Fig. 2). Likewise, the serum 8-OHG levels in patients who had advanced nephropathy (azotemia) (9.31 ± 0.38 pmol/ml) were higher than in patients with normoalbuminuria (3.54 ± 0.28 pmol/ml), microalbuminuria (5.74 ± 0.55 pmol/ml), or overt proteinuria (3.40 ± 0.09 pmol/ml) (Fig. 3).

CONCLUSIONS — The nonenzymatic, free radical–mediated oxidation of biological molecules is associated with a variety of pathologic events, such as cancer, aging, and diabetes (17). In diabetes, the role of active oxygen species has been demonstrated as a cause of type 1 diabetes induced by chemicals such as alloxan and streptozotocin in experimental animals (18,19). Not only are oxygen radicals involved in the cause, but diabetic status itself is associated with increased production of free radicals, and this condition in turn has been suggested as one of the pathogenetic mechanisms of complications (20,21). In our study, using serum 8-OHG as a marker, we demonstrated that type 2 diabetic patients have significantly increased oxidative DNA damage compared with control subjects. This result is in agreement with an earlier report by Dandona et al. (11), who observed a higher concentration of 8-OH-dG in mononuclear cells of type 1 and type 2 diabetic patients compared with control subjects.

We used serum 8-OHG, rather than 8-OH-dG, as a biomarker of oxidative DNA damage. Although 24-h urinary 8-OH-dG levels have been used for the measurement of oxidative stress, deoxyguanosine may be oxidized during the work-up procedure for DNA as well as during the preparation for analysis, resulting in false high levels of 8-OH-dG (15). Moreover, the convenience of collecting serum as opposed to 24-h urine sampling is another advantage, especially when it is necessary to screen a large number of samples.

In our study, considerable overlap in serum 8-OHG levels was found between control subjects and diabetic patients. In addition, hyperglycemia, which has been implicated as a major factor for enhanced free radical production in diabetic patients, was not related to serum 8-OHG. In this regard, it is of note that no correlation between blood glucose level and urinary 8-OH-dG level was found in the study of Dandona et al. (11). These results are quite unexpected, but suggest that hyperglycemia per se is not linked to DNA oxidation in diabetic subjects. The level of 8-OHG is known to be influenced by a series of confounding factors. It is higher in cigarette smokers, in men compared with women, in lean subjects, in inflammatory states, etc. (12). Recently, it has also been proposed that alteration in intracellular lipid metabolism, which frequently coexists with and precedes diabetes, may be a cause of increased oxidative stress (22). Finally, the lack of

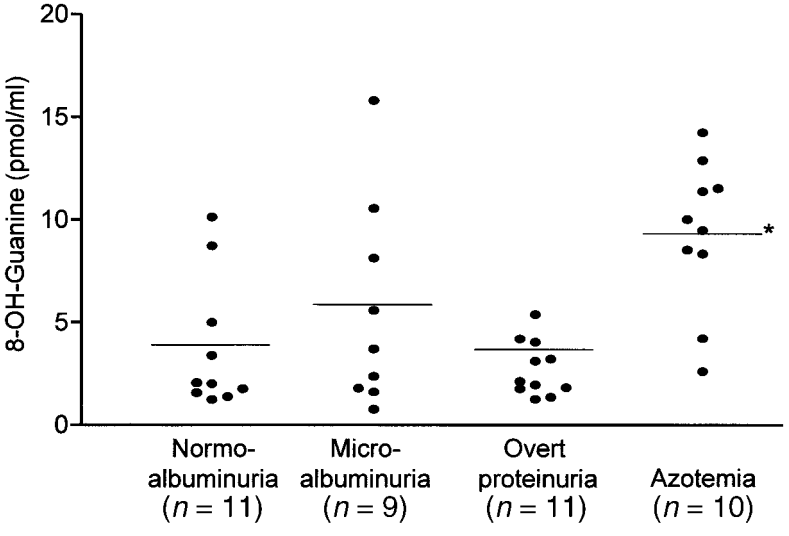

Figure 3—*Serum 8-OHG levels in type 2 diabetic patients according to diabetic nephropathy group. *P < 0.01 vs. normoalbuminuria, microalbuminuria, and overt proteinuria. The line represents mean value.*

association between hyperglycemia and serum 8-OHG could be explained by the fact that 8-OHG levels are related to renal function. However, there was only a marginal correlation between 8-OHG and creatinine clearance, suggesting that the contribution of renal impairment to the increased 8-OHG levels may not be very strong.

Of interest in our study is that serum 8-OHG levels are increased in the patients with PDR and advanced nephropathy. Although little is known about the participation of oxidative stress in retinopathy, Grattagliano et al. (23) demonstrated an increase in MDA and carbonyl proteins and a decrease in vitamin E and sulfhydryl proteins in subretinal fluid of diabetic patients, especially in those patients with PDR. Similarly, in streptozotocin-induced diabetic rats, tissue levels of 8-OH-dG in kidneys were significantly higher than those of control rats and paralleled urinary albumin excretion (24). Plasma (25) or erythrocyte (26) MDA content has been shown to be higher in type 2 diabetic patients with nephropathy than in patients without nephropathy. In addition, administration of ROS scavenger to streptozotocin-induced diabetic animals decreased MDA as well as albuminuria without affecting glycemic control. (27). The present study, showing increased serum 8-OHG levels in advanced diabetic microvascular complications, supports the aforementioned idea that increased oxidative stress contributes to the development of diabetic microvascular complications. However, because of the cross-sectional nature of our study, firm conclusions regarding the causal relationship cannot be drawn.

Increased serum 8-OHG levels in diabetic patients 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. Lee et al. (28) reported that 18% of Korean type 2 diabetic patients with known duration <5 years already had microalbuminuria caused by a long unrevealed duration of diabetes. Thus, the known duration of diabetes may not reflect the real duration of disease in some patients (29).

In conclusion, serum 8-OHG concentration in type 2 diabetic patients was significantly increased compared with that of control subjects, and 8-OHG concentration was even higher in the patients with advanced complications. Although hyperglycemia per se is less likely to have contributed to the increase in 8-OHG levels, these results allow us to conclude that diabetic patients show greater oxidative damage to DNA, which might play a role in the pathogenesis of diabetic complications. One should be cautious in interpreting these results, however, because our study is not able to tell the causal relationship between oxidative stress and the development of complications. Moreover, oxidative injury is likely to be dependent on the balance between free radical generation and antioxidant defense of the tissue. Further studies should investigate the role of antioxidant treatment in type 2 diabetic patients as a potential therapy for the prevention of diabetic complications.

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