Evaluation of Urinary 8-Hydroxydeoxyguanosine as a Novel Biomarker of Macrovascular Complications in Type 2 Diabetes

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OBJECTIVE — To evaluate urinary 8-hydroxydeoxyguanosine (8-OHdG) as a marker for the progression of diabetic macroangiopathic complications.

RESEARCH DESIGN AND METHODS — The content of urinary 8-OHdG, common carotid intima-media thickness (IMT), the coronary heart disease (CHD) risk score, the severity of diabetic retinopathy, and urinary albumin excretion were examined in 96 patients with type 2 diabetes, including 32 patients who had been nominated for the Kumamoto Study [Shichiri M, et al. Diabetes Care 23 (Suppl 2):B21–B29, 2000]. In addition, the patients from the Kumamoto Study were further evaluated regarding the effect of intensive insulin therapy on urinary 8-OHdG excretion.

RESULTS — The urinary 8-OHdG/creatinine ratio (U8-OHdG) was 2.5-fold higher in patients with increased HbA1c than in those with normal HbA1c (P < 0.05). In addition, U8-OHdG was 2.3-fold higher in patients with increased IMT and CHD risk score (P < 0.01). U8-OHdG was significantly higher in patients with simple retinopathy (P < 0.05) and those with advanced retinopathy (P < 0.01) than in patients without retinopathy. Similarly, U8-OHdG was significantly higher in patients with albuminuria (P < 0.01). Furthermore, in the Kumamoto Study, U8-OHdG was significantly lower in the multiple insulin injection therapy group compared with the conventional insulin injection therapy group (P < 0.01).

CONCLUSIONS — Hyperglycemia independently increases 8-OHdG in patients with type 2 diabetes. 8-OHdG is a useful biomarker of not only microvascular but also macrovascular complications in patients with type 2 diabetes.

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vascular complications are the leading cause of morbidity and mortality in patients with diabetes. In adult patients with diabetes, the risk of cardiovascular disease is three- to fivefold greater than in nondiabetic subjects despite controlling for other known risk factors for cardiovascular disease (1). In addition, diabetic microangiopathy still represents one of the main causes of blindness (2), terminal renal failure (3), and amputation (4).

The outcomes of the Diabetes Control and Complication Trial (5), the Kumamoto Study (6–8), and the U.K. Prospective Diabetes Study (9) seem to have effectively resolved the long debate over whether chronic hyperglycemia is an important cause of diabetic vascular complications. Furthermore, the Diabetes Insulin–Glucose in Acute Myocardial Infarction Study showed that intensive insulin treatment was associated with a lower mortality rate than conventional insulin treatment in subjects with acute myocardial infarction (10). It was also reported that early atherosclerosis could be retarded by improved glycemic control in patients with type 1 diabetes (11). Therefore, hyperglycemia represents a major contributing factor to not only microvascular complications in diabetes but also macrovascular complications. Next to this, the longest-running controversy yet to be resolved concerns the identification of the mechanisms through which hyperglycemia acts as a crucial risk factor for these vascular complications.

Production of reactive oxygen species (ROS) and lipid peroxidation are increased in diabetic patients, especially in those with poor glycemic control (12). Oxidative stress may be crucial for development of diabetic vascular complications. Recently, we have shown that normalizing levels of mitochondrial ROS (MROS) prevents the three major pathways known as the causes of hyperglycemic damage: glucose-induced activation...
8-OHdG and diabetic macrovascular complications

Table 1—Clinical characteristics of type 2 diabetic patients

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Diet</th>
<th>Oral hypoglycemic agents</th>
<th>CIT</th>
<th>MIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (men/women)</td>
<td>5/7</td>
<td>20/13</td>
<td>11/8</td>
<td>19/13</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.2±9.8</td>
<td>64.2±11.3</td>
<td>65.3±9.4</td>
<td>60.1±9.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.1±7.4</td>
<td>24.3±7.5</td>
<td>22.5±10.0</td>
<td>23.5±6.8</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>10.7±7.8</td>
<td>14.0±9.0</td>
<td>23.5±9.4</td>
<td>18.1±12.6</td>
</tr>
</tbody>
</table>
| Mean HbA1c (%)             | 6.5±1.2 | 7.8±1.3               | 8.2±1.5 | 7.4±1.1*
| Smoking (yes/no)           | 6/6  | 20/13                    | 9/10 | 12/20|
| Systolic blood pressure (mmHg) | 127.1±15.3 | 133.2±15.4        | 139.6±13.9 | 124.8±15.7*|
| Total cholesterol (mg/dl)  | 208.3±33.7 | 193.5±45.7           | 212.1±45.0 | 192.4±38.9|
| HDL cholesterol (mg/dl)    | 61.8±20.8 | 51.6±14.7            | 56.3±13.8 | 63.4±20.8|
| Mean IMT (mm)              | 1.33±0.62 | 1.57±0.54            | 1.64±0.67 | 1.26±0.61*
| CHD score                  | 15.9±13.8 | 17.4±8.6             | 20.8±11.5 | 13.0±8.7*
| U8-OHdG (µg/g creatinine)  | 37.9±29.6 | 70.3±58.8            | 91.2±76.1 | 37.2±35.0*|

Data are means ± SD. Mean values for HbA1c were calculated during the previous year. *P < 0.05 vs. CIT.

of protein kinase C, increased formation of glucose-derived advanced glycation end products, and increased glucose of glucose-derived advanced glycation of protein kinase C, increased formation/H1106 Data are means

of Kumamoto Study, the salutiary effect of intensive therapy on microvascular complications was explained to all patients included in the study and multiple insulin injection therapy (MIT) was recommended to the patients who had been treated with CIT. However, some patients still continued CIT for personal reasons. Therefore, in this study, 32 patients who had been nominated for the Kumamoto Study were still being treated with MIT or CIT (18 and 14 patients, respectively) after the end of the study. All of patients fulfilled the World Health Organization (WHO) diagnostic criteria for type 2 diabetes (14). Patients with disseminated cancer or a reduced life expectancy were excluded from the study group. Informed consent was obtained from each patient. In each patient, HbA1c was measured every month. The mean value for HbA1c was calculated to evaluate glycemic control during the previous year. The characteristics of the patients are shown in Table 1.

RESEARCH DESIGN AND METHODS

Patients
The study group consisted of 96 outpatients with type 2 diabetes at the Hospital of Kumamoto University School of Medicine, including 32 patients who had been nominated for the Kumamoto Study during 1987–1998 (6–8). The Kumamoto Study was a randomized clinical trial designed to compare intensive insulin therapy using multiple insulin injections with conventional insulin injection therapy (CIT) to evaluate the effects of glycemic control on the development and progression of microvascular complications in Japanese patients with type 2 diabetes. After the end of Kumamoto Study, the salutor of oxidative DNA damage to the pathogenesis of diabetic micro- and macroangiopathic complications, we measured the content of urinary 8-OHdG and mean intima-media thickness (IMT) of carotid arteries and calculated the cormonary heart disease (CHD) risk score in 96 patients with type 2 diabetes.

Measurement of IMT of common carotid artery
The left and right common carotid arteries were examined in the anterior-oblique, lateral, and posterior-oblique longitudinal projections with an echocardiographic system (LOGIQ 500; GE Yokogawa Medical System, Tokyo, Japan), as reported by Kawamori et al. (15).

CHD risk score
A venous blood sample was collected after an overnight fast. Plasma total and HDL cholesterol were measured with commercial enzymatic kit (Kyowa Medex, Tokyo, Japan). CHD risk score was assigned to each risk indicator, including age, total and HDL cholesterol, systolic and diastolic blood pressure, cigarette smoking, and diabetes, as described (16).

Classification of retinopathy
All of the patients underwent direct ophthalmoscopy and photography with pupils dilated. The eyes were graded as no retinopathy, simple retinopathy, or either preproliferative or proliferative retinopathy using the Davis classification (17).

Measurement of 8-OHdG, creatinine, and albumin in urea
A morning urine sample from each patient was collected and stored frozen under N₂ gas at –70°C. Urine samples were used within 1 month for the determination of 8-OHdG using a competitive ELISA kit (8-OHdG Check; Japan Institute for the Control of Aging, Shizuoka, Japan) according to the manufacturer’s instructions. The contents of creatinine and albumin in the same sample were measured using the Creatinine-Test (Wako, Osaka, Japan) and the Albuwell (Exocell), respectively.

Statistical analysis
Data were expressed as means ± SD. Differences between two groups were compared using the Mann-Whitney U test. Multiple comparisons were performed using the Kruskal-Wallis test followed by Scheffe’s test. Correlations between variables were tested using the Spearman rank-correlation analysis. P < 0.05 was considered statistically significant.
RESULTS — As shown in Fig. 1A, the urinary 8-OHdG:creatinine ratio (U8-OHdG) was 2.5-fold higher in patients with increased HbA1c than in those with normal HbA1c (63.6 ± 59.7 and 26.6 ± 13.7 μg/g creatinine in HbA1c > 5.8 and <5.8%, respectively; \( P < 0.05 \)). Multivariate analysis was performed between increased U8-OHdG and other cardiovascular disease risk factors, including sex, age, BMI, duration of diabetes, mean HbA1c, smoking, systolic blood pressure, and total and HDL cholesterol. Age, mean HbA1c, and smoking are independently associated with increased U8-OHdG (Table 2).

As shown in Fig. 1B, the patients were divided into two groups according to their value of mean IMT (>1.1 or <1.1 mm). U8-OHdG was 2.3-fold higher in the high IMT group than in the normal IMT group (71.8 ± 80.0 μg/mg creatinine).

Table 2—Possible contributing factors of the increased U8-OHdG or mean IMT in type 2 diabetic patients

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>U8-OHdG</th>
<th>Mean IMT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard partial</td>
<td>( F ) (Probability)</td>
</tr>
<tr>
<td></td>
<td>regression coefficient</td>
<td></td>
</tr>
<tr>
<td>Sex (men = 1, women = 0)</td>
<td>-0.024</td>
<td>0.055 (0.8)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.208</td>
<td>4.108 (0.05)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.143</td>
<td>2.133 (0.1)</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>0.070</td>
<td>0.531 (0.3)</td>
</tr>
<tr>
<td>Mean HbA1c (%)</td>
<td>0.347</td>
<td>11.821 (0.001)</td>
</tr>
<tr>
<td>Smoking (yes = 1, no = 0)</td>
<td>0.209</td>
<td>4.458 (0.04)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.124</td>
<td>1.601 (0.2)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>0.065</td>
<td>0.488 (0.5)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>-0.114</td>
<td>1.318 (0.3)</td>
</tr>
<tr>
<td>U8-OHdG (μg/g creatinine)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are means ± SD. Mean values for HbA1c were calculated from the previous year.
63.0 vs. 30.7 ± 25.1 μg/g creatinine, respectively; \( P < 0.005 \)). Similarly, a significant positive correlation existed between U8-OHdG and CHD risk score (\( r = 0.27, P < 0.01 \); Fig. 1C). Multivariate analysis between increased IMT and the cardiovascular disease risk factors sex, age, BMI, duration of diabetes, mean HbA1c, smoking, systolic blood pressure, total and HDL cholesterol, and U8-OHdG demonstrated that age, U8-OHdG, and decreased HDL cholesterol are independent risk factors for increased IMT or higher CHD risk score. In addition, Kawamori et al. (15) reported that diabetic patients were divided into three groups according to severity of retinopathy: no retinopathy, simple retinopathy, or either preproliferative or proliferative retinopathy. U8-OHdG was significantly higher in patients with simple retinopathy (77.2 ± 74.0 μg/g creatinine, \( P < 0.05 \)) and those with either preproliferative or proliferative retinopathy (68.9 ± 51.9 μg/g creatinine, \( P < 0.01 \)) than in patients without retinopathy (25.8 ± 13.5 μg/g creatinine). However, there was no significant difference between patients with simple retinopathy and those with either preproliferative or proliferative retinopathy.

The relationship between the severity of diabetic nephropathy and U8-OHdG was evaluated, as shown in Fig. 1D. The patients were divided into three groups according to the severity of nephropathy: no nephropathy, simple nephropathy, or either micro- or macrovascular nephropathy. U8-OHdG was significantly higher in patients with simple nephropathy (27.9 ± 10.1 μg/g creatinine) and those with either micro- or macrovascular nephropathy (48.9 ± 29.1 μg/g creatinine, \( P < 0.01 \)) than in patients without nephropathy (9.1 ± 6.2 μg/g creatinine). However, there was no significant difference between patients with simple nephropathy and those with either microvascular or macrovascular nephropathy.

CONCLUSIONS — The present study shows the positive correlation between U8-OHdG and either HbA1c, mean IMT, or CHD risk score in type 2 diabetic patients. In addition, we showed that U8-OHdG increased in patients with albuminuria and in those with retinopathy. These data suggest that U8-OHdG is a useful marker of early micro- and macrovascular complications in type 2 diabetic patients and that increased oxidative stress could play an important role in the progression of diabetic complications. U8-OHdG is a product of oxidative DNA damage following specific enzymatic cleavage after 8-hydroxylation of the guanine base. U8-OHdG increases with aging (19), during carcinogenesis (20), during radiotherapy (21), in smokers (22), and in patients with diabetes (23). Recently, the content of U8-OHdG in the urine and that of the isolated mononuclear cells from type 2 diabetic patients with either retinopathy or nephropathy were reported to be much higher than those in patients without these complications (24). A similar trend was observed in our results of the association between U8-OHdG and diabetic retinopathy or nephropathy, although we could not find significant difference of U8-OHdG between the patients with simple retinopathy and those with advanced retinopathy. Therefore, it is suggested that U8-OHdG could be a sensitive biomarker especially for the patients with early stages of diabetic complications.

The detection of early changes of atherosclerosis by noninvasive and quantitative methods is mandatory. Several cross-sectional community-based studies and follow-up studies have shown a strong and graded association between increased IMT and increased incidences of CHD and stroke (25). In addition, Kawamori et al. (15) reported that diabetic patients showed increased mean IMT compared with nondiabetic subjects and suggested that mean IMT was also useful in evaluating clinical manifestations of atherosclerosis in diabetic individuals. The CHD risk score reported by Framingham Heart Study was assigned to risk indicators, including age, HDL and total cholesterol, systolic and diastolic blood pressure, cigarette smoking, and diabetes, allowing estimation of an individual’s 10-year risks of CHD in a population free from CHD at baseline (16). This point score has been used to classify each patient’s vascular risk (16). Increased mean IMT of the carotid arteries and CHD risk score are considered to be useful markers of atherosclerosis, even in diabetic patients. In the present study, we show that U8-OHdG is elevated in patients with either increased IMT or higher CHD risk score. In addition, multivariate analysis shows that U8-OHdG, age, and decreased HDL cholesterol are independent risk factors for increased IMT. In accordance with our results, Taniwaki et al. (26) reported that age and decreased HDL cholesterol were the independent risk factors for increased IMT in type 2 diabetic patients. However,

![Figure 2 — The effect of intensive insulin therapy on the U8-OHdG and IMT in type 2 diabetic patients from the Kumamoto Study. Values are means ± SD. The values of the U8-OHdG and IMT in patients treated by MIT or CIT in the Kumamoto Study are shown in A and B, respectively.](image-url)
to the best of our knowledge, there is no other report to show the association between IMT and U8-OHdG.

Increased oxidative stress has been considered to be one of the common pathogenic factors of diabetic complications. However, the mechanisms by which hyperglycemia increases the production of ROS are not fully understood. Recently, we have shown a hypothesis that MROS is a main cause of the progression of diabetic complications in vivo. In the present study, we show that mean HbA1c is independently associated with U8-OHdG in type 2 diabetic patients and 8-OHdG is a useful biomarker of not only microvascular but also macrovascular complications in patients with type 2 diabetes. It has been reported that the steady-state burden of oxidative adducts, such as 8-OHdG, was 16-fold higher in mtDNA than in nuclear DNA in rat liver (27). Furthermore, it has been reported that a 4,977-bp deletion in mitochondrial DNA and 8-OHdG content in muscle DNA increased in proportion to the severity of diabetic retinopathy or nephropathy in type 2 diabetic patients (24). Because 8-OHdG is a product of oxidative DNA damage and most 8-OHdG is generated in mitochondria, these findings suggest that hyperglycemia induces MROS, which contributes, at least in part, to the pathogenesis of diabetic complications in vivo.

The Kumamoto Study was a randomized clinical trial designed to compare MIT and CIT to evaluate the effects of glycemic control on the development and progression of microvascular complications in Japanese patients with type 2 diabetes (6–8). In the Kumamoto Study, we reported that intensive glycemic control can delay the onset and progression of early stages of diabetic microvascular complications. However, there is still considerable controversy with respect to the precise mechanism by which hyperglycemia contributes to the development of macrovascular complications. In the present study, we found that U8-OHdG was significantly lower in the MIT group compared with the CIT group in some patients from the Kumamoto Study after more than 10 years of insulin therapy. Furthermore, we showed that mean IMT was significantly less in the MIT group than in the CIT group. Although we never knew the content of U8-OHdG or the value of mean IMT in those patients at commencement of the Kumamoto Study, these findings suggest that intensive glycemic control could normalize or reduce oxidative stress and consequently delay the onset and progression of early stages of diabetic micro- and macrovascular complications. Further prospective follow-up study will be required to determine whether intensive glycemic control can actually decrease oxidative DNA damage in diabetic patients.

In conclusion, hyperglycemia independently increases 8-OHdG in type 2 diabetic patients and 8-OHdG is a useful biomarker of not only microvascular but also macrovascular complications in patients with type 2 diabetes. Blocking intracellular ROS formation, through mitochondrial electron transport chains, could offer an additional strategy for the potential prevention of diabetic micro- and macrovascular complications, which deserves further exploration.

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References


