

New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus

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Abstract Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) has been reported to serve as a sensitive biomarker of oxidative DNA damage and also of oxidative stress. We have investigated oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus (NIDDM) by urinary 8-OHdG assessments. We determined the total urinary excretion of 8-OHdG from 24 h urine samples of 81 NIDDM patients 9 years after the initial diagnosis and of 100 non-diabetic control subjects matched for age and gender. The total 24 h urinary excretion of 8-OHdG was markedly higher in NIDDM patients than in control subjects ($68.2 \pm 39.4 \mu\text{g}$ vs. $49.6 \pm 37.7 \mu\text{g}$, $P = 0.001$). High glycosylated hemoglobin was associated with a high level of urinary 8-OHdG. The increased excretion of urinary 8-OHdG is seen as indicating an increased systemic level of oxidative DNA damage in NIDDM patients.

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Key words: Oxidative stress; Oxidative DNA damage; 8-Hydroxy-2'-deoxyguanosine; Diabetes mellitus, non-insulin-dependent

1. Introduction

Reactive oxygen species (ROS) and oxidative stress may be crucial for the development of the vascular complications of diabetes [1–3]. Hyperglycemia leads to autooxidation of glucose [2] and causes consequent glycooxidation of proteins as well as low-density lipoprotein (LDL). This is, in turn, associated with vascular changes and atherosclerosis [2,4].

8-Hydroxy-2'-deoxyguanosine (8-OHdG) is a product of oxidative DNA damage following specific enzymatic cleavage after 8-hydroxylation of the guanine base. 8-OHdG increases in plasma with aging [5], during the development of tumors caused by ROS [6], during radiotherapy [7] and in smokers [8]. Urinary 8-OHdG is a new putative biomarker of the total systemic oxidative stress in vivo [9,10].

Mononuclear cells of diabetic patients have recently been reported to contain a high level of 8-OHdG [11], but there are overall very few data on oxidative DNA damage in patients

with diabetes. Thus, we have measured urinary 8-OHdG in a cohort of non-insulin-dependent diabetes mellitus (NIDDM) patients 9 years after diagnosis.

2. Materials and methods

The study group consisted of 81 NIDDM outpatients at the municipal health care center of the City of Tampere. The patients fulfilled the WHO diagnostic criteria for NIDDM. One hundred non-diabetic attendants of the same health care center, matched for age and gender, were used as control subjects. All subjects gave written informed consent. The study was approved by the ethics committees of the Tampere University Hospital and the health care center of the City of Tampere. The recruitment of patients and control subjects and the diagnostic criteria have been previously discussed in detail [12]. Subjects with disseminated cancer and a reduced life expectancy were excluded from both groups. Some biochemical and demographic characteristics of the study groups are presented in Table 1. At the time of study 11 patients were treated with diet alone, 47 patients with an oral antidiabetic drug, and 23 patients were on insulin.

2.1. Methods

All blood samples were collected after an overnight fast. Serum cholesterol and triglycerides were determined by the dry slide technique (Ektachem 700 analyzer, Johnson and Johnson Clinical Diagnostics, Rochester, NY, USA). High-density lipoprotein (HDL) cholesterol was measured with the same technique after precipitation of LDL. Blood glucose, glycosylated hemoglobin (HbA_{1c}) and urinary albumin were determined by routine laboratory methods. The glomerular filtration rate (GFR) was determined by the plasma clearance of [⁵¹Cr]EDTA assessed by the single injection method [13]. Urinary creatinine was measured with Creatinine-Test (Wako, Osaka, Japan). The body mass index (BMI) was calculated as body weight (kg)/height² (m). A doctor asked about cigarette smoking (yes/no at present time).

A 2 ml sample of a well-mixed 24 h urine collection was stored frozen at -70°C until analyzed. Urine samples were centrifuged at $10000 \times g$ for 10 min, and after proper dilution the supernatant was used for the determination of 8-OHdG by a competitive ELISA (8-OHdG Check, Japan Institute for the Control of Aging, Fukuroi, Shizuoka). The determination range was 0.64–2000 ng/ml. The specificity of the monoclonal antibody N45.1 used in the competitive ELISA kit has been established [14]. Urinary 8-OHdG is expressed as the total amount excreted in 24 h. The urinary 8-OHdG/creatinine ratio was alternatively used in analysis with consistent results.

Analysis of covariance (ANCOVA) was used to compare 8-OHdG excretion between groups. The dependent factors in ANCOVA were NIDDM, smoking and gender, and BMI was used as a covariate. Pearson's correlation matrix was used for correlation analysis. Computations were carried out using Statistica for Windows ver. 5.0 (Statsoft Inc., Tulsa, OK, USA). Data in the text are given as mean $\pm$

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Table 1
Biochemical, clinical and demographic characteristics of the study groups

	NIDDM patients	Control subjects
Gender (male/female, <i>n</i>)	49/32	54/46
Age (years)	64.6 ± 7.5	65.0 ± 6.9
Smokers (<i>n</i>)	8 (9.9%)	18 (18.0%)
Duration of disease (years)	9.4 ± 0.8	–
BMI (kg/m ²)	30.0 ± 5.3*	27.7 ± 4.5
Fasting blood glucose (mM)	9.3 ± 3.0*	4.9 ± 1.0
Fasting serum insulin (IU)	31.7 ± 76.2*	11.8 ± 6.9
HbA _{1c} (%)	8.3 ± 1.6*	5.7 ± 0.7
Triglycerides (mM)	2.0 ± 1.3*	1.5 ± 0.8
Total cholesterol (mM)	5.3 ± 1.0*	5.7 ± 1.2
HDL cholesterol (mM)	1.1 ± 0.4	1.2 ± 0.4
Blood pressure (mm Hg, syst/diast)	162 ± 21*/88 ± 9	153 ± 20/87 ± 8
24 h urinary albumin (mg)	97.0 ± 215*	21.3 ± 53.7
GFR (ml/min/1.73 m ²)	97.7 ± 23.4	90.5 ± 19.7

Results are means ± S.D. * $P \leq 0.05$ between groups.

standard deviation (S.D.). A P value less than 0.05 was considered significant.

3. Results

The total 24 h urinary 8-OHdG excretion was 68.2 ± 39.4 μg in NIDDM patients and 49.6 ± 37.7 μg in control subjects ($P = 0.001$) (35.9 ± 16.4 vs. 24.3 ± 15.2 ng/mg creatinine, respectively, $P < 0.0001$). In three-way ANCOVA the impact of smoking and gender weakened this difference between diabetic patients and control subjects (Fig. 1). A detailed analysis showed that male subjects had a markedly higher urinary excretion of 8-OHdG than the corresponding female subjects (80.8 ± 44.6 vs. 48.9 ± 16.8 μg , $P = 0.0002$, in diabetic patients,

and 62.4 ± 42.0 vs. 34.6 ± 24.9 μg , $P = 0.0001$, in control subjects). Smoking increased the urinary 8-OHdG excretion only in the control subjects, and thus the difference between diabetic patients and control subjects was diminished in smokers (Fig. 1).

The patients were divided into three groups according to their level of HbA_{1c}. The group in poorest glycemic control included patients with HbA_{1c} in the upper quartile, i.e. patients with HbA_{1c} higher than 9.6%, the second group comprised patients with HbA_{1c} between 6.8% and 9.6%, while the patient group in best glycemic control had HbA_{1c} in the lower quartile, i.e. less than 6.8%. In one-way ANCOVA there were significant differences in the level of urinary 8-OHdG between these groups ($P = 0.04$) (Fig. 2). The group of patients in poorest glycemic control had total urinary excretion of 8-OHdG 56% higher than the group in best glycemic control ($P = 0.009$) and 77% higher than the control subjects ($P = 0.00004$). On the other hand, the difference in urinary 8-OHdG between patients in best glycemic control and control subjects was not statistically significant. The same effect of blood glucose control on urinary 8-OHdG was also observed separately in male, female, and non-smoking patients. The total 24 h urinary excretion of 8-OHdG had a weak positive correlation with GFR both in NIDDM patients ($r = 0.28$, $P = 0.03$) and in control subjects ($r = 0.29$, $P = 0.02$), but no correlation with 24 h urinary albumin was observed. Urinary 8-OHdG also had no significant correlations with BMI, blood cell count, blood pressure, serum fasting insulin, or lipid related variables.

4. Discussion

Urinary 8-OHdG is a new index of systemic oxidative DNA damage that has been repaired [15]. 8-OHdG passes freely into the urine by glomerular filtration and serves as an index of whole body oxidative stress [9]. Immunological assessment of 8-OHdG yields comparable results to the HPLC-based method [16], which is more commonly used, but is more convenient for non-invasive studying of large numbers of urine samples. However, the metabolism of 8-OHdG has not been thoroughly elucidated in humans and some of the 8-OHdG may arise from deoxyGTP, a DNA precursor base [17]. It is not established whether urinary 8-OHdG originates from living or dead cells [18], although secretion from living cells is supported. All mammalian cells contain enzymatic activity

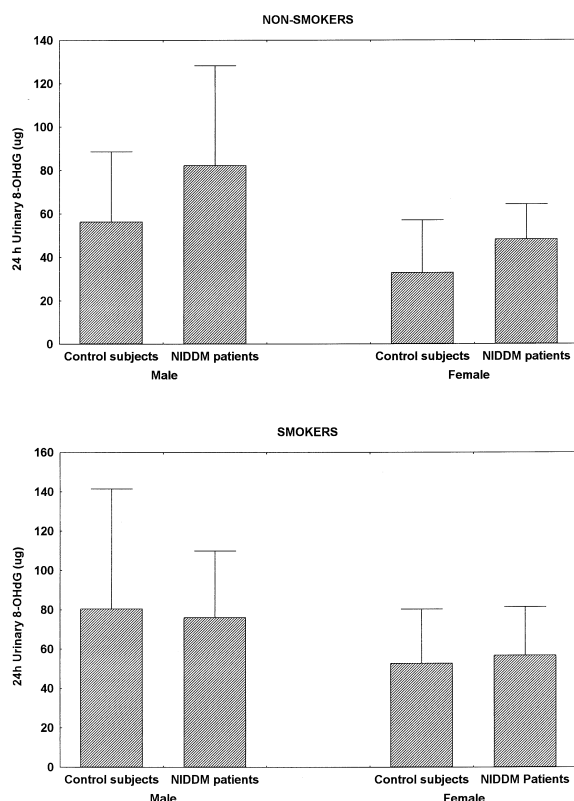


Fig. 1. 24 h urinary 8-OHdG excretion in NIDDM patients and control subjects by gender and smoking. * $P < 0.01$ between diabetic patients and control subjects.

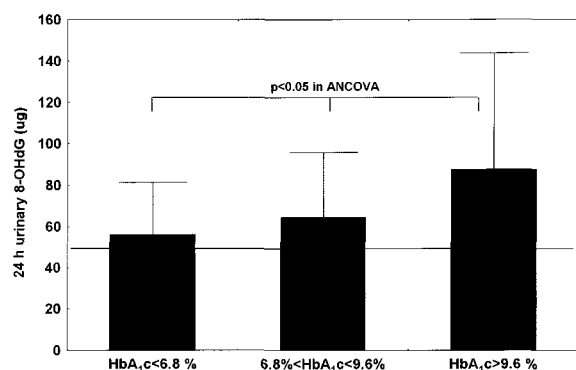


Fig. 2. The 24 h urinary excretion of 8-OHdG in NIDDM patients according to the level of HbA_{1c} (mean±S.D.). The horizontal line represents the mean 24 h urinary excretion of 8-OHdG of control subjects.

specifically cleaving 8-OHdG-containing DNA [19]. The diet apparently does not affect the urinary excretion of 8-OHdG [20].

The present study indicates an increased oxidative DNA damage in NIDDM patients 9 years after diagnosis. Additionally, the highest excretion level of urinary 8-OHdG was seen in male smokers. The difference in urinary 8-OHdG between patients and control subjects was also diminished in smoking subjects. However, the small number of smokers ($n=8$) among NIDDM patients prevented reliable analysis of the possible additive effect of smoking on urinary 8-OHdG. Poor glycemic control was associated with a high urinary excretion of 8-OHdG. This finding is in good agreement with the idea of oxidative stress in NIDDM originating from constant hyperglycemia. An association between poor glycemic control in NIDDM and oxidative stress has been previously demonstrated and discussed [1,2,4,21,22].

Oxidative DNA damage and 8-OHdG have been extensively studied in relation to the development of cancer [6,8]. In diabetes mellitus, however, there is no increased risk for cancer, despite the increased systemic DNA damage implied by the high excretion of 8-OHdG in this study. Oxidative DNA damage has, however, been shown to be related to the peroxidation of membrane fatty acids and low antioxidant status [23], both present in diabetes.

In conclusion, we found high urinary excretion of 8-OHdG in NIDDM patients, especially in patients with poor blood glucose control. The full meaning of this increased systemic oxidative DNA damage in diabetes is unknown and requires further elucidation.

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