Association of serum 8-hydroxy-2′-deoxyguanosine levels with the presence and severity of coronary artery disease

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Objective **Oxidative stress is involved in the pathogenesis of atherosclerosis. 8-Hydroxy-2′-deoxyguanosine (8-OHdG), an oxidative product of DNA, is a sensitive biomarker to reflect oxidative stress status *in vivo*. However, the circulating 8-OHdG levels in patients with coronary artery disease (CAD) have not yet been elucidated. The purpose of this study is to investigate whether serum 8-OHdG levels are associated with the presence and severity of CAD.**

Methods **We measured serum 8-OHdG levels by enzyme-linked immunosorbent assay in 127 patients with suspected CAD who underwent coronary angiography. The severity of CAD was assessed by the number of diseased vessels and Gensini score.**

Results **The serum 8-OHdG levels in patients with CAD were significantly higher than those in patients with normal coronary arteries [median (interquartile range), 0.41 (0.30–0.57) ng/ml versus 0.32 (0.25–0.43) ng/ml, $P=0.001$]. Log (8-OHdG) levels increased with the number of diseased vessels ($P=0.002$) and significantly correlated with Gensini score ($r=0.379$, $P=0.001$). The multivariable logistic regression analysis showed that serum 8-OHdG was independently associated with the presence of CAD (odds ratio, 1.318; $P=0.027$).**

Conclusion **In conclusion, the serum 8-OHdG levels are increased in patients with CAD and are associated with the severity of coronary artery stenosis. 8-OHdG might serve as an independent factor for predicting CAD. *Coron Artery Dis* 22:223–227 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: atherosclerosis, coronary artery disease, 8-hydroxy-2′-deoxyguanosine, oxidative stress

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Introduction

Oxidative stress induced by reactive oxygen species (ROS) contributes to cell damage, proliferation, endothelial dysfunction, and oxidation of low-density lipoproteins, and plays a prominent role in the onset and progression of atherosclerosis and coronary artery disease (CAD) [1–3]. It is difficult to directly measure the oxidative stress status *in vivo*, therefore, measurement of the products of oxidative modified molecules, such as DNA, lipids, and proteins, is more feasible to assess oxidative stress status [4].

ROS can alter deoxyguanosine, one of the constituents of DNA, into 8-hydroxy-2′-deoxyguanosine (8-OHdG), which is ultimately released into blood and excreted through urine after being excised from DNA by the repair enzyme system. 8-OHdG is one of the major oxidative products of DNA, which is regarded as a reliable marker of oxidative DNA damage. Furthermore, earlier studies have shown that there are positive correlations between 8-OHdG levels and other well-established markers of oxidative stress, such as malondialdehyde [5,6] and 8-iso-prostaglandin F2α [7]. Therefore, 8-OHdG has been widely used as a sensitive biomarker of oxidative stress [8]. Moreover, high levels of 8-OHdG have been identified in the human atherosclerotic plaques and in peripheral blood lymphocytes in patients with CAD [9,10]. However, the circulating 8-OHdG levels in patients with CAD have not yet been elucidated. In this study, we aimed to investigate whether serum 8-OHdG levels are associated with the presence and severity of CAD assessed by coronary angiography (CAG).

Methods and materials

Patients

Patients selected to participate in the study were 127 consecutive patients who had undergone CAG with suspected CAD, recruited from the Cardiology Department of Sun Yat-sen Memorial Hospital of Sun Yat-sen University. Exclusion criteria were having other heart diseases including valvular heart disease, cardiomyopathy, severe chronic heart failure (New York Heart Association functional class III or IV), acute coronary syndrome within 30 days, and a history of percutaneous coronary intervention or coronary artery bypass surgery. Moreover, patients with acute or chronic inflammatory disease, immunological disease, renal or hepatic dysfunction, history

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or presence of neoplastic disease, and age over 80 years were also excluded. All patients were asked for their medical history and the presence of coronary risk factors, such as smoking habits, hypertension, diabetes mellitus, and hyperlipidemia. The traditional coronary risk factors were assessed according to following definitions: hypertension (current systolic blood pressure $\geq$140 mmHg and/or diastolic blood pressure $\geq$90 mmHg, or use of antihypertensive drugs), hyperlipidemia (fasting total cholesterol $\geq$220 mg/dl, or triglyceride $\geq$200 mg/dl, or with hypolipidemic drug therapy), diabetes mellitus (fasting blood glucose $\geq$126 mg/dl, or treatment with oral hypoglycemic agents or insulin), and smoking (current smokers who smoked $\geq$10 cigarettes per day or stopped smoking <6 months ago). No patients were receiving antioxidant therapy. The study protocol was approved by the local ethics committees, and informed consent was obtained from each patient.

Coronary angiography

All patients underwent CAG using standard Judkins technique. The coronary angiograms were analyzed by two experienced angiographers who were unaware that the patients were enrolled in the study. Significant CAD was defined as the presence of luminal diameter stenosis greater than or equal to 50% in one or more main coronary arteries, and normal coronary artery was defined as without any stenosis. Patients with CAD were further divided to three subgroups by the number of involved coronary arteries [1-vessel disease (VD), 2-VD, and 3-VD] to determine the severity of CAD. In addition, the extent of CAD was also estimated by means of Gensini scoring system, which provided an index of the severity of CAD by calculating the Gensini score according to the degree of luminal narrowing and lesion location [11].

Measurement of serum 8-hydroxy-2′-deoxyguanosine levels

Venous blood samples were collected in the fasting state before angiographic procedures. After centrifugation at 3000 rpm for 20 min, the samples were stored at -80°C until assay. Determination of serum 8-OHdG concentration was achieved by a competitive enzyme-linked immunosorbent assay kit (Japan Institute for the Control of Aging, Fukuroi, Shizuoka, Japan) according to the manufacturer's instructions. Before the assay procedures, samples were pretreated by centrifugal ultrafiltration, using ultrafilter (Microcon YM-10, Millipore, Bedford, Massachusetts, USA) to remove large molecular weight substances, which could disturb the measurement. The measuring range of the 8-OHdG kit was 0.125–10 ng/ml.

Statistical analysis

Data were tested for normal distributions with the Kolmogorov–Smirnov test, and because the 8-OHdG values are skewed distribution, a logarithmic transformation was used for statistical analysis. Variables are expressed as mean $\pm$ standard deviation or median (interquartile range), or percentage. Comparisons between two groups for study variables were made by using the Student's *t*-test, Mann–Whitney *U*-test, or the χ^2 test as appropriate. Comparisons among multiple groups were made by one-way analysis of variance. The relationship between continuous variables was analyzed using Pearson correlation coefficient or Spearman correlation analysis according to data distributions. Multivariable logistic regression analysis was used to determine the independent factors associated with the presence of CAD. All statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, Illinois, USA), and a *P* value of less than 0.05 was considered statistically significant.

Results

Of the total 127 patients, 74 patients had significant CAD (CAD+) and 53 patients had normal CAD (CAD−). The clinical and laboratory characteristics of the two groups are summarized in Table 1. Compared with CAD− group, the mean age and the proportion of male patients were higher in the CAD+ group. The presentation of patients with hypertension, hyperlipidemia, and diabetes mellitus in the CAD+ group was more compared with the CAD− group.

To examine the relationship between circulating 8-OHdG and CAD, we measured serum 8-OHdG levels in all 127 patients. We found that the serum 8-OHdG levels were significantly higher in patients with CAD compared with those without CAD [0.41 (0.30–0.57) vs. 0.32 (0.25–0.43) ng/ml, $P = 0.001$] (Table 1). In addition, serum Log (8-OHdG) levels were correlated with age ($r = 0.190$, $P = 0.033$), and patients with hypertension showed higher Log (8-OHdG) levels than patients without hypertension (-0.39 ± 0.20 vs. -0.48 ± 0.22, $P = 0.032$).

Table 1 Clinical and laboratory characteristics of patients with and without coronary artery disease

	CAD+ (*N*=74)	CAD− (*N*=53)	*P*
Male sex (%)	47 (63.5)	24 (45.3)	0.041
Smoking (%)	24 (32.4)	11 (20.8)	0.146
Hypertension (%)	57 (77.0)	30 (56.6)	0.015
Hyperlipidemia (%)	41 (55.4)	19 (35.8)	0.029
Diabetes mellitus (%)	26 (35.1)	10 (18.9)	0.045
Age (years)	63.38 ± 8.64	57.19 ± 9.04	<0.001
Total cholesterol (mg/dl)	199.1 ± 46.2	193.5 ± 33.1	0.423
LDL cholesterol (mg/dl)	121.0 ± 40.1	112.7 ± 30.5	0.164
HDL cholesterol (mg/dl)	44.3 (36.2–51.7)	48.1 (38.9–56.4)	0.070
Triglycerides (mg/dl)	147.5 (107.2–258.7)	138.2 (101.0–169.2)	0.282
hs-CRP (mg/dl)	2.28 (1.17–4.75)	1.60 (0.83–2.97)	0.041
LVEF (%)	62.68 ± 12.54	65.45 ± 11.89	0.738
Serum 8-OHdG (ng/ml)	0.41 (0.30–0.57)	0.32 (0.25–0.43)	0.001
Log (8-OHdG)	−0.37 ± 0.22	−0.50 ± 0.18	0.001

Data are expressed as mean ± standard deviation or median (interquartile range), or percentage.
CAD, coronary artery disease; CAD+, significant CAD; CAD−, normal CAD; HDL, high-density lipoprotein; hs-CRP, high-sensitive C-reactive protein; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction.

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No statistically significantly different Log (8-OHdG) levels were observed in patients with or without other coronary risk factors.

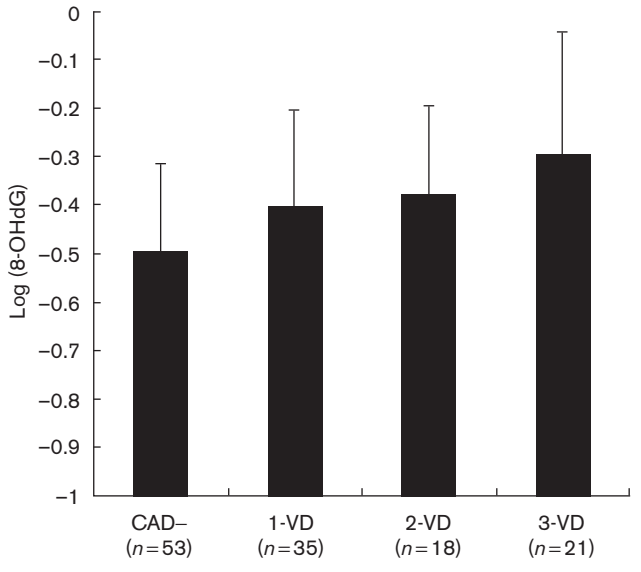
In the CAD + group, 35 had 1-VD, 18 had 2-VD, and 21 had 3-VD. Log (8-OHdG) levels of the three subgroups are -0.40 ± 0.20, -0.38 ± 0.19, and -0.30 ± 0.25 for 1-VD, 2-VD, and 3-VD, respectively. Log (8-OHdG) levels continuously increased with the number of diseased vessels ($P = 0.002$ for trend) (Fig. 1). Furthermore, correlation analysis showed that Log (8-OHdG) levels were significantly correlated with Gensini score ($r = 0.379$, $P = 0.001$) (Fig. 2).

Multivariable logistic regression analysis in the model including male sex, smoking, hypertension, hyperlipidemia, diabetes mellitus, age, and serum 8-OHdG levels showed that serum 8-OHdG levels were independently associated with the presence of CAD. Serum 8-OHdG concentration with 0.1 ng/ml increase was associated with an odds ratio of 1.318 (95% confidence interval, 1.032–1.682; $P = 0.027$) for the presence of CAD (Table 2).

Discussion

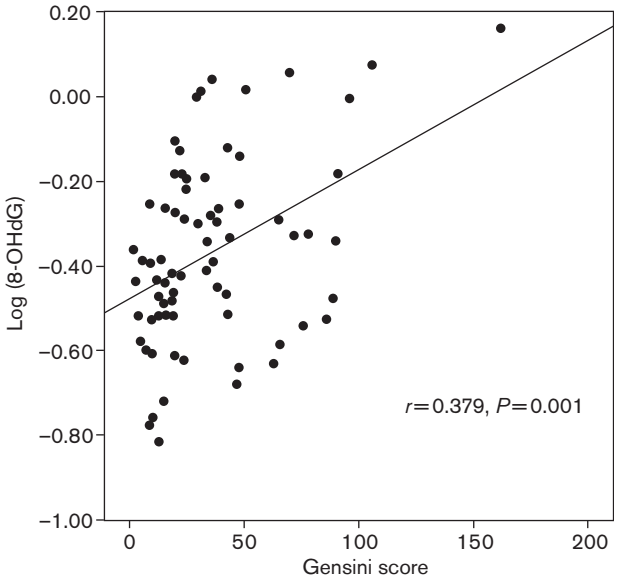
This study has shown for the first time that the serum 8-OHdG levels were associated with the severity of coronary artery stenosis evaluated by the number of diseased vessels and Gensini score. Moreover, the serum 8-OHdG was found to be an independent factor associated with the presence of CAD.

Fig. 1



Log [8-hydroxy-2′-deoxyguanosine (8-OHdG)] levels in the normal coronary artery disease (CAD−) group and patients with CAD categorized according to the number of diseased vessels. VD, vessel disease. $P = 0.002$ for trend.

Fig. 2



Correlation of Log [8-hydroxy-2′-deoxyguanosine (8-OHdG)] with Gensini score in significance coronary artery disease group.

Table 2 Multivariable logistic regression analysis for presence of coronary artery disease in all patients

	OR	95% CI	*P*
Male sex	2.472	0.919–6.648	0.073
Smoking	2.104	0.675–6.561	0.200
Hypertension	1.590	0.621–4.067	0.333
Hyperlipidemia	2.429	1.012–5.827	0.047
Diabetes mellitus	3.851	1.308–11.340	0.014
Age (+10 years)	2.457	1.474–4.097	0.001
8-OhdG (+0.1 ng/ml)	1.318	1.032–1.682	0.027

CI, confidence interval; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; OR, odds ratio.

Excessive ROS are generated in patients with CAD. They have high chemical reactivity and can attack many macromolecules *in vivo*, including DNA, lipids, and proteins. Oxidative damage products of these substances are used to assess oxidative stress status. Earlier studies have shown the relationship between CAD and the oxidative products of lipids and proteins, such as malondialdehyde [12], 8-iso-prostaglandin F2α [13], protein carbonyl [12], and advanced oxidation protein products [14]. DNA oxidative products, especially 8-OHdG or its tautomer 8-oxo-7-hydro-2′-deoxyguanosine, were also studied in atherosclerosis and CAD. Increased 8-OHdG levels were shown in human carotid atherosclerotic plaques [9,15]. Other reports showed that the levels of 8-OHdG in peripheral blood lymphocytes were elevated in patients with CAD and correlated with severity of the disease [10,12]. Recently, Nagayoshi *et al.* [16] reported that urinary 8-OHdG levels were related to the severity of CAD in patients with chronic

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heart failure. This study confirmed that serum 8-OHdG levels can indicate the severity of CAD, and these observations suggest that 8-OHdG level may be one potential biomarker of CAD.

In fact, compared with lipid or protein oxidative products, the DNA oxidative products may have some advantages in study on atherosclerosis, because the DNA damage itself contributes to atherosclerosis development [17]. In the 'monoclonal hypothesis', atherosclerotic plaque formation was caused by the mutation of a single smooth muscle cell proliferation through cloning formed special smooth muscle cells. According to this hypothesis, alterations at the DNA level may be involved in the development and progression of atherosclerosis [18]. Growing evidences have shown that various DNA abnormalities, such as chromosomal aberrations [19], loss of heterozygosity and microsatellite instability [20], micronucleus [21], and DNA strand breaks [12] were present in atherosclerotic patients. In this perspective, furthermore, 8-OHdG might be not only just a biomarker of oxidative stress, but also itself may take part in the pathogenesis of atherosclerosis, as 8-OHdG has a mutagenic effect, leading to G→T and A→C transition mutations [22]. Therefore, detailed investigation of 8-OHdG might provide more valuable information in atherosclerosis study.

Consistent with previous studies [5,23], we found that the high 8-OHdG levels were associated with age and hypertension in this population. It is known that oxidative stress is elevated in these two and other atherogenic risk factors, which might induce atherosclerosis partly by their effects on increasing systemic oxidative stress [24,25].

This study has several limitations that require consideration. First, our sample size was relatively small and it is a cross-sectional study, thus large sample and prospective studies are expected to confirm our findings and show the potential value of 8-OHdG for predicting future cardiovascular events. Another limitation is that some cardiovascular drugs, which some patients used, might affect oxidative status and 8-OHdG levels [26,27], but we did not exclude these patients or assess the influence of drugs on the 8-OHdG levels in this study.

In conclusion, this study has shown the important role of oxidative stress in the development of atherosclerosis. Serum 8-OHdG levels are associated with the presence and severity of CAD, and 8-OHdG might serve as a potential predictor for CAD.

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