Asian Biomedicine Vol. 6 No. 5 October 2012; 759-764
DOI: 10.5372/1905-7415.0605.118

Brief communication (Original)

Significance of serum 8-Hydroxydeoxyguanosine levels to the intima-media thickness of the carotid artery in type 2 diabetes

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Background: Cardiovascular disease is the largest cause of morbidity and mortality in patients with type 2 diabetes mellitus. Interestingly, previous studies demonstrated that type 2 diabetic patients had a significantly higher concentration of 8-Hydroxydeoxyguanosine (8-OHdG) although it role in the pathogenesis of carotid artery atheroma and insulin resistance has not been identified.
Objective: We investigated the role of serum 8-OHdG levels in the intima-media thickness (IMT) of the carotid artery, and its significance in insulin resistance in type 2 diabetes.
Methods: Fifty patients with disease-onset type 2 diabetes were enrolled and divided into two groups according to the intima-media thickness of the carotid artery (<1.0 mm = group A or ≥1.0 mm = group B). Blood samples from patients were used within one month for the determination of 8-OHdG using a competitive ELISA kit, and other coronary heart disease (CHD) risk factors were also measured.
Results: Patients in Group B showed significant higher levels of serum 8-OHdG compared to that of group A and control. Serum 8-OHdG had significant positive correlations with BMI, SBP, FPG, FINS, HbA1c, TC, and Homa-IR. Similarly, a significant positive correlation existed between the serum 8-OHdG and IMT levels. 8-OHdG, Homa-IR, HbA1c and TC were independently associated with increased IMT in type 2 diabetes patients.
Conclusion: Serum 8-OHdG is a useful indicator for the severity of IMT of the carotid artery and reflects the severity of insulin resistance in type 2 diabetes.

Keywords: Carotid artery, insulin resistance, intima-media thickness, type 2 diabetes, 8-hydroxydeoxyguanosine

Type 2 diabetes is the most common non-communicable disease on a global scale and is being fueled by the worldwide obesity epidemic. Currently, there are 285 million people worldwide living with diabetes, and 90 to 95% have type 2 diabetes. This number is expected to reach 439 million by 2030 [1]. The prevalence of type 2 diabetes mellitus continues to climb in many parts of the globe in association with the rise in obesity. Although the latter is clearly a predominant factor in the pathogenesis of type 2 diabetes, other modifiable lifestyle factors such as exercise, alcohol consumption, smoking, and certain nutritional factors, such as vitamin D deficiency, are also believed to play a role [2].

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Cardiovascular disease (CVD) is the largest cause of morbidity and mortality in patients with type 2 diabetes mellitus (T2DM) [3]. Insulin resistance is a risk factor for diabetes, which is also an important risk factor for CVD [4]. Although there is an abundance of evidence suggesting that insulin resistance plays a significant role in the vasculature, the precise mechanistic role involved remains unclear [5]. Understanding the role of various nutritional or other modifiable risk factors that may contribute to the pathogenesis of diabetes and impaired glucose tolerance is important in the effort to combat the rising tide of diabetes and CVD worldwide.

Recent studies suggest that insulin resistance, diabetes, and CVD all share a common involvement with oxidative stress. The production of reactive oxygen species (ROS) is increased in diabetic patients, especially in those with poor glycemic control [6]. Excessive ROS can accelerate oxidative damage to macromolecules, including lipids and proteins, as well

as to DNA. 8-Hydroxydeoxyguanosine (8-OHdG), a ROS-induced modification of a purine residue in DNA, is a sensitive index of oxidative DNA damage [7]. Previous studies demonstrated that type 1 and type 2 diabetic patients had a significantly higher concentration of 8-OHdG in their mononuclear cells as well as urinary samples [8, 9]. However, the role of serum 8-OHdG in the pathogenesis of carotid artery atheroma and insulin resistance has not been identified.

This study was undertaken to investigate whether the serum levels of 8-OHdG were altered in type 2 diabetic patients. We also attempted to analyze the relationship between 8-OHdG levels and intima-media thickness (IMT) of carotid artery as well as insulin resistance in type 2 diabetes patients.

Materials and methods

Patients

The study group consisted of 50 newly-diagnosed type 2 diabetes patients at the First Affiliated Hospital of Suzhou University, who had no obesity. All patients fulfilled the diagnostic criteria for type 2 diabetes proposed by the World Health Organization (WHO) [10], aged 48.9±7.3 years (36 to 64 years), with body mass index (BMI) of 22.72±1.30 kg/m^2 (19.05 to 24.86 kg/m^2), and had no history of smoking or hypertension. None of the subjects was taking any anti-inflammatory or antioxidant drugs that would have affected the assay of the serum indexes. Patients were divided into two groups according to the thickness of IMT [11]: group A (n = 32), unilateral or bilateral IMT <1.0 mm; group B (n = 18), unilateral or bilateral IMT ≥1.0 mm, or with confirmed carotid sclerosis plaque. Twenty-five age- and sex-matched healthy individuals were served as controls (group C), aged 46.2±6.5 years (35 to 62 years), with BMI of 22.22±1.91 kg/m^2 (18.49 to 24.56 kg/m^2). The characteristics of the patients were shown in **Table 1**.

Written informed consent was obtained from all subjects, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Suzhou University. The trial was conducted in compliance with current Good Clinical Practice standards and in accordance with the principles set forth under the Declaration of Helsinki (1989).

Measurement of serum 8-OHdG

A morning blood sample from each patient was collected and stored at -70°C. Blood samples were used within 1 month for the determination of 8-OHdG using a competitive ELISA kit (8-OHdG Check; Trevigen, USA) following the manufacturer's instructions.

Measurement of carotid 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 echotomographic system (SONOS5500, HP, USA), with probe frequency ranging from 7.0 to 11.0 MHz. For each patient, 4-point IMT values were evaluated by a specified operator, including the thickness of both anterior and posterior walls 1 cm distant from the crotch of bilateral common carotid artery. The detection was repeated five times, and the IMT was evaluated as the mean of 20 values.

CHD risk score

Serum fasting blood glucose (FBG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglyceride (TG) were measured using the enzymatic technique on an autoanalyzer (Olympus 2007). Fasting insulin levels (FINS) were detected by ELISA kit (DSL, USA). Tissue insulin sensitivity was calculated by homeostasis model assessment using the following formula: Homa-IR = FPG (mmol/L) × FINS (U/ml)/22.5, as reported previously [12]. Glycosylated hemoglobin (HbA1c) was measured by cation exchange microcolumn chromatography. The height, body weight, and blood pressure were also measured for each patient.

Statistical analysis

Data were expressed as means±SD. All statistical analyses were performed using the statistical software SPSS version 13.0. Differences between groups were compared by the one-way ANOVA test, unpaired *t* test or Mann-Whitney *U* test. Correlations between variables were tested using the Spearman rank-correlation analysis. Multiple regression analysis was used to investigate the risk factors for IMT. $p < 0.05$ was considered statistically significant.

Results

Serum 8-OHdG elevated in patients with higher IMT

There were no significant differences of age, BMI, SBP, DBP, TG, HDL-c, and LDL-c among the three

groups (p >0.05). Compared with the normal individuals, patients in both groups A and B showed elevated FPG, FINS, HbA1c, Homa-IR and IMT (**Table 1**). Additionally, patients in groups A and B showed much higher levels of serum 8-OHdG compared with that in group C (3.25±0.46 ng/ml in group A, 4.12±1.62 ng/ml in group B, 2.16±0.68 ng/ml in group C (**Figure 1A**). Moreover, patients in group B had significantly higher levels of Homa-IR compared with that in group A (**Figure 1B**). All the data suggested that serum 8-OHdG might participate in the pathogenesis of Homa-IR and facilitate higher IMT in type 2 diabetes patients.

Correlation between serum 8-OHdG and clinical indexes

Spearman rank-correlation analysis demonstrated that serum 8-OHdG had significantly positive correlations with BMI, SBP, FPG, FINS, HbA1c, TC, and Homa-IR (p <0.05). Similarly, a significant positive correlation existed between the serum 8-OHdG and IMT levels in patients with type 2 diabetes (r = 0.712, p <0.001) as shown in **Figure 2**.

Table 1. Demographic characteristics of the patients

Characteristics	Group A	Group B	Group C
Number	32	18	25
Age (years)	48.66±7.20	49.44±7.75	46.16±6.49
BMI (kg/m^2)	22.75±1.29	22.66±1.36	22.22±1.91
SBP (mmHg)	126±10	126±14	120±13
DBP (mmHg)	79±9	82±9	76±9
FPG (mmol/L)	9.28±2.52[a]	9.36±2.45[a]	4.87±0.50
FINS (mu/L)	6.51±3.48	12.80±3.89[ab]	8.14±3.85
HbA1c (%)	9.98±1.64[a]	10.15±1.66[a]	5.29±0.77
TG (mmol/L)	1.68±1.16	2.14±2.04	1.21±0.72
TC (mmol/L)	4.42±1.24	5.19±1.41[ab]	4.26±1.02
HDL-c (mmol/L)	1.24±0.30	1.19±0.35	1.34±0.26
LDL-c (mmol/L)	3.11±0.78	3.04±0.75	2.84±0.78
IMT (mm)	0.56±0.24[a]	1.63±0.44[ab]	0.30±0.18
HOMA-IR	6.51±3.48[a]	12.8±3.9[ab]	8.14±3.85

a = p <0.05 *vs.* group C, b = p <0.05 *vs.* group A.

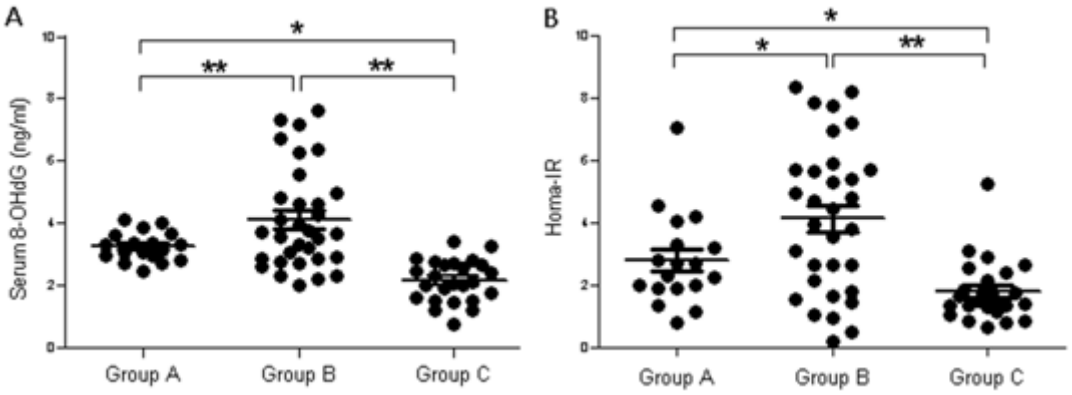

Figure 1. Patients in both groups A (with IMT<1.0mm) and group B (with IMT ≥1.0 mm) showed significant higher levels of serum 8-OHdG compared with the healthy controls in group C. *p <0.05, **p <0.01. IMT, intima-media thickness; 8-OHdG, 8-Hydroxydeoxyguanosine.

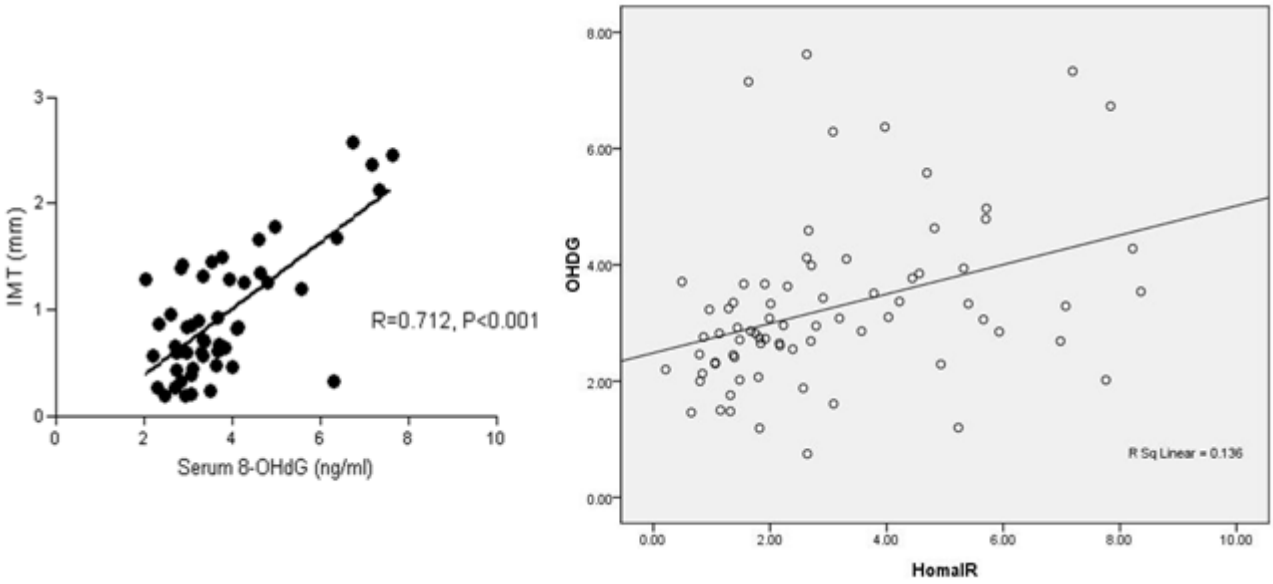

Figure 2. Serum 8-OHdG concentrations demonstrated significant positive correlation with IMT in patients with type 2 diabetes. *p <0.05, **p <0.01. IMT, intima-media thickness; 8-OHdG, 8-Hydroxydeoxyguanosine.

Risk factors for IMT in type 2 diabetes patients

Non-normally distributed and irregular variances were logarithmically transformed. Multivariate regression analysis between increased IMT and the risk factors of cardiovascular diseases including age, BMI, SBP, DBP, HbA1c, Homa-IR, TC, TG, HDL-C, LDL-C, and 8-OHdG demonstrated that 8-OHdG, Homa-IR, HbA1c and TC were independently associated with increased IMT in type 2 diabetes patients (**Table 2**), with an equation of Y = -3.477+ $0.619X_{\text{ln8-OHDG}}+0.325X_{\text{lnHoma IR}}+0.615X_{\text{lnHbA1c}}+0.128X_{\text{T}}$.

Discussion

The present study revealed the positive correlation between 8-OHdG and mean IMT, HbA1c, Homa-IR, and risk factors of CHD in type 2 diabetic patients. In addition, the results demonstrated that serum 8-OHdG was a risk factor for IMT. It is suggested that serum 8-OHdG is a useful marker of early micro- and macro-vascular complications in newly-diagnosed type 2 diabetic patients and that increased oxidative stress plays an important role in the severity of diabetic complications.

Oxidative stress develops from an imbalance between oxidant production and antioxidant activity in cells and plasma. This overabundance of oxidants is associated with the multifactorial etiology of insulin resistance, primarily in skeletal muscle tissue [13, 14]. Correlative evidence in humans indicates an association between plasma markers of oxidative stress and damage and the degree of insulin resistance [15]. In the current study, a significantly positive correlation was found between the levels of serum 8-OHdG, a product of DNA damage following specific enzymatic cleavage, and Homa-IR in patients with newly-onset type 2 diabetes, which demonstrated the intrinsic role of oxidative stress product in patients.

Table 2. Equation for multiple liner regression

Variant	*B* (partial regression coefficient)	Beta (standardized partial regression coefficient)	*t*	*p*
Constant	-3.477	-	-5.958	0.000
ln8-OHDG	0.619	0.294	2.778	0.018
lnHoma-IR	0.325	0.265	2.826	0.006
lnHbA1c	0.615	0.232	2.183	0.032
TC	0.128	0.178	1.748	0.085

In addition to its documented contributions to the etiology of insulin resistance in type 2 diabetes, oxidative stress has been implicated in the development of diabetic complications, including diabetic retinopathy [16], nephropathy [17], and peripheral neuropathy [18]. Nevertheless, in this study, we focused on the role of 8-OHdG in cardiovascular complication in type 2 diabetes patients. The detection of early changes of atherosclerosis by noninvasive and quantitative methods is of great importance for clinical application. 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 [19]. In addition, other studies reported that diabetic patients showed increased mean IMT compared with the non-diabetic subjects, and proved that mean IMT was also useful in evaluating clinical manifestations of atherosclerosis in diabetic individuals [20]. This point score has been used to classify the vascular risk in each patient [21]. Increased mean IMT of the carotid arteries and CHD risk score are considered to be useful markers of atherosclerosis, especially in diabetic patients. In the present study, the results showed that serum 8-OHdG was elevated in patients with increased IMT. We also found significant positive correlations between serum 8-OHdG and Homa-IR and CHD risk factors. Furthermore, multivariate regression analysis demonstrated that serum 8-OHdG was a risk factor for IMT. It is indicated that serum 8-OHdG may be used as a biomarker for occurrence of IMT in type 2 diabetes patients.

The detailed mechanism of oxidative stress to the development of atherosclerosis has not been clearly identified. Firstly, redundant ROS induces cascade activation of serine/threonine kinase signaling pathway, which then interferes with the insulin signaling pathway and reduces the insulin sensitivity [13]. Secondly, oxidative stress induces insulin resistance by attenuating the transportation and translation of glucose transporter 4 (GluT4) [22]. Thirdly, ROS induces lipid peroxidation and causes damage to the endothelial cells, and then platelets aggregate and enhance the LDL intake potential of vessel wall, leading to acceleration of atherosclerosis by inducing and priming the formation of foam cells [23, 24].

In conclusion, serum concentration of 8-OHdG can be used as a biomarker for the degree of insulin resistance and cardiovascular complication in type 2 diabetes patients.

Acknowledgement

This work was supported by grants from Natural Science Foundation of China (No.81170174), Natural Scientific Fund of Jiangsu Province (BK2011304), Social development Fund of Suzhou (SYSD2011081) and Jiangsu Province's Key Provincial Talents Program (RC2011111). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Xingbo Cheng and Yafeng Zhou have contributed equally to this study, and should be considered as co-corresponding authors. The authors declare no conflict of interest to report.

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