



Increased 8-hydroxy-2'-deoxyguanosine in leukocyte DNA from patients with type 2 diabetes and microangiopathy

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Abstract

Objective: To evaluate oxidative damage in leukocytes from patients with type 2 diabetes by examining 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels.

Methods: Patients with type 2 diabetes and healthy controls were assessed for demographic, clinical and biochemical characteristics. Levels of 8-OHdG in extracted leukocyte DNA were determined by enzyme linked immunosorbent assay.

Results: Of 108 patients with type 2 diabetes (56 with microangiopathy, 52 without) and 65 healthy controls, leukocyte 8-OHdG levels were higher in patients with type 2 diabetes versus controls (median \pm interquartile range [IQR], 3.19 ± 2.17 versus 0.38 ± 1.00 ng/ml), and higher in patients with type 2 diabetes and microangiopathy versus those without microangiopathy (median \pm IQR, 3.34 ± 1.87 versus 2.71 ± 2.26 ng/ml). Patients with type 2 diabetes and microangiopathy had higher serum creatinine and urinary albumin levels versus those without microangiopathy. Leukocyte 8-OHdG levels, duration of type 2 diabetes, albuminuria, use of insulin and use of angiotensin-converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARBs) were independently associated with microangiopathy in patients with type 2 diabetes after adjustment for smoking.

Conclusions: Leukocyte oxidative DNA damage was high in patients with type 2 diabetes and microangiopathy. Presence of microangiopathy was associated with leukocyte 8-OHdG levels, duration of type 2 diabetes, albuminuria and use of ACE inhibitors/ARBs or insulin.

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Keywords

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Introduction

Type 2 diabetes is an endocrine disorder characterized by insulin resistance and deficiency, resulting in chronic hyperglycaemia that may lead to renal, neurological and cardiovascular complications.¹ Obesity and age are risk factors for type 2 diabetes.¹ Long-term vascular complications are the main cause of type 2 diabetes-associated morbidity and mortality.¹ In 2008, worldwide prevalence of type 2 diabetes was 9.8% in males and 9.2% in females;² in 2010, prevalence of type 2 diabetes in the Chinese population was reported to be 11.6%.³

Metabolic dysfunctions associated with type 2 diabetes include dyslipidaemia,⁴ oxidative stress⁵ and activated leukocytes.⁶ Oxidative stress and leukocyte activation have been associated with the development of type 2 diabetes complications.⁵⁻⁷ Oxidative stress may lead to cell damage and dysfunction through the free-radical-mediated decomposition of vital molecules (particularly DNA, which lacks chemical stability);⁸ oxidative stress has been shown to activate nuclear transcription factor (NF)- κ B, leading to DNA damage.⁹ Studies have shown increased oxidative DNA damage¹⁰⁻¹² and accumulation of 8-hydroxy-2'-deoxyguanosine (8-OHdG)¹¹⁻¹³ in patients with type 2 diabetes, thus suggesting the involvement of hyperglycaemia in oxidative DNA damage. A product of oxidative DNA damage following specific enzymatic cleavage after 8-hydroxylation of the guanine base, 8-OHdG is considered to be a good marker of oxidative DNA damage that can easily be measured using an enzyme linked immunosorbent assay (ELISA).¹⁴

Microangiopathy in patients with type 2 diabetes is associated with increased platelet and leukocyte activation;¹⁵ however, specific leukocyte DNA damage and factors involved in this damage are poorly understood. The authors of the present study hypothesized that leukocyte DNA is damaged in type 2 diabetes, and that an advanced diabetic state (i.e. with microangiopathy) is associated with further DNA damage. Therefore, the aim of the study was to evaluate the extent of oxidative DNA damage in leukocytes from patients with type 2 diabetes and microangiopathy, by examining peripheral blood leukocyte 8-OHdG levels, in an attempt to provide further insights into the pathogenesis of microangiopathy and indications for treatment.

Patients and methods

Study population

This case-control study included sequentially enrolled patients with type 2 diabetes recruited at the Department of Endocrinology, Jinling Hospital, Nanjing, China, between October 2007 and April 2008. All patients had been diagnosed according to World Health Organization criteria.¹⁶ Inclusion criteria were: (i) aged 40–70 years; (ii) no history of coronary artery disease, cerebral infarction or peripheral vascular diseases, as shown by Holter monitoring, Doppler ultrasound, magnetic resonance imaging, computed tomography and/or coronary angiography; (iii) no antioxidant therapy; (iv) no lipid-lowering therapy in the 6 months prior to study entry; (v) no history of angina or myocardial infarction (normal 12-lead resting electrocardiograms and normal peripheral Doppler studies); (vi) no confirmed atherosclerosis,

vascular stenosis or occlusion in the lower extremities as shown by Doppler ultrasound.

Healthy controls were recruited from individuals who consulted for routine physical examinations at the Health Examination Centre, Jinling Hospital. Inclusion criteria for controls were: (i) aged 40–70 years; (ii) no personal or family history of hypertension, diabetes, coronary diseases or kidney diseases; (iii) no drug use in the 6 months prior to study entry (including pain medications, e.g. over-the-counter acetaminophen or non-steroidal anti-inflammatory drugs, and dietary supplements, e.g. vitamins).

Microangiopathy was diagnosed if nephropathy or retinopathy, or both, were present. Nephropathy was diagnosed if patients had either persistent proteinuria (≥ 500 mg/day) or persistent microalbuminuria (albuminuria estimated by the albumin:creatinine ratio > 30 $\mu\text{g}/\text{mg}$) in the absence of urinary tract infection.¹⁷ Retinopathy was assessed using stereoscopic colour retinal photography as previously described.¹⁸ Hypertension was diagnosed if patients were being treated with antihypertensive drugs or had a systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg. Patients with diabetes without microangiopathy were diagnosed based on the absence of retinopathy (assessed by retinal photography) or nephropathy (24 h protein excretion < 100 mg/day and albumin : creatinine ratio < 30 $\mu\text{g}/\text{mg}$).

The study was approved by the ethics committee of Nanjing Jinling Hospital. Written informed consent was obtained from all study participants.

Clinical parameters and laboratory measurements

All participants underwent a clinical and physical (including height, weight and body mass index [BMI]) examination, and demographic and clinical data were recorded.

Blood pressure was recorded in the right arm while participants were seated.

Two readings were taken 5 min apart and the mean was used for analysis. Blood samples (5 ml) were taken following an overnight fast. Serum was separated by leaving samples to clot for 1 h at room temperature. For plasma collection, blood samples were drawn into ethylenediaminetetra-acetic acid (EDTA) anti-coagulation tubes and plasma was separated by centrifugation at 400 *g* for 5 min at room temperature. Samples were stored at -70°C prior to use. Biochemical analyses were performed on a Hitachi 7600 Autoanalyser (Hitachi, Tokyo, Japan) and included fasting plasma glucose (Glucose Kit, TCI, Japan), serum total cholesterol (Total Cholesterol test kit, Randox Laboratories Ltd, County Antrim, UK), serum triglycerides (Triglycerides test kit, Randox Laboratories Ltd) and high-density lipoprotein cholesterol (HDL-C; HDL Cholesterol test kit, Randox Laboratories Ltd). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula ($[\text{LDL-C}] = [\text{Total cholesterol}] - [\text{HDL-C}] - ([\text{TG}]/2.2)$). Glycosylated haemoglobin (HbA_{1c}) was estimated by high-pressure liquid chromatography (HLC-723G8; TOSOH, Tokyo, Japan), according to the manufacturer's instructions. Samples were processed with the hospital's usual clinical samples; laboratory staff were thus blinded to the study groups.

Leukocyte DNA 8-OHdG measurements

Venous blood samples (10 ml drawn into EDTA anti-coagulation tubes) were obtained from participants following an overnight fast. Leukocyte DNA was extracted from peripheral blood samples within 1 h of blood collection, using PureGene™ reagents (SBS Genetech Co. Ltd, Shanghai, China) according to the manufacturer's protocol, which included separation of the nucleated cells from whole blood and the salting-out method.¹⁹ DNA purity was determined

using absorbance at 260/280 nm and absorbance at 260/230 nm. Isolated DNA was stored at -80°C prior to use. For each sample, 200 μg DNA was dissolved in 135 μl of nuclease free water, then 15 μl (200 mM) sodium acetate and 15 μl (6 units) nuclease P1 (Sigma, St Louis, MO, USA) were added to the DNA solution and incubated for 30 min at 37°C . Following incubation, 15 μl Tris-HCl buffer (1 M, pH 7.4) and 7 μl (2 units) alkaline phosphatase (Takara, Tokyo, Japan) were added and incubated for another 30 min at 37°C . The hydrolysate was filtered through a Millipore Microcon[®] Centrifugal Filter (Merck Millipore, Darmstadt, Germany) at 1000g centrifugation for 10 min, and then 50 μl of each digested DNA sample

was assessed using a Highly Sensitive 8-OHdG Check ELISA kit (JaICA, Fukuroi, Shizuoka, Japan), according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microtitre plate reader (JaICA, Fukuroi, Shizuoka, Japan). Results were expressed in ng/ml, then 1 ng/ml was converted to 4.8 8-OHdG/ 10^6 dG, based on a previously described method.²⁰ Samples were assayed in a blind manner.

Statistical analyses

All statistical analyses were performed using SPSS[®] version 11.0 (SPSS Inc., Chicago, IL, USA). Normally distributed continuous data are presented as mean \pm SD, and were

Table 1. Demographic and clinical characteristics of Chinese patients with type 2 diabetes and healthy controls.

Variable	Study group		Statistical significance
	Healthy controls n = 65	Type 2 diabetes n = 108	
Age, years	55 \pm 11	60 \pm 17	NS
Sex, male	36 (55.4)	67 (62.0)	NS
Duration of diabetes, months	0	98.02 \pm 77.68	–
Fasting plasma glucose, mmol/l	4.6 \pm 1.1	10.4 \pm 3.3	$P < 0.001$
HbA _{1c} , %	5.5 \pm 0.7	9.0 \pm 2.9	$P < 0.001$
BMI, kg/m ²	22.5 \pm 3.2	23.5 \pm 5.4	NS
SBP, mmHg	114 \pm 28	126 \pm 39	NS
DBP, mmHg	70 \pm 14	79 \pm 27	NS
Total cholesterol, mmol/l	3.22 \pm 0.88	4.74 \pm 1.67	$P = 0.030$
Triglycerides, mmol/l	1.44 \pm 0.76	1.67 \pm 1.20	NS
LDL cholesterol, mmol/l	1.77 \pm 0.58	2.83 \pm 0.85	$P = 0.012$
HDL cholesterol, mmol/l	1.13 \pm 0.24	1.24 \pm 0.21	NS
Serum creatinine, mg/dl	0.82 \pm 0.19	0.91 \pm 0.31	NS
Albuminuria, mg/24 h	9.22 \pm 3.44	141.92 \pm 116.36	$P < 0.001$
8-OHdG, ng/ml	0.38 \pm 1.00	3.19 \pm 2.17	$P < 0.001^{\text{a}}$
Smoker, yes	19 (29.2)	27 (25.0)	NS

Data presented as mean \pm SD, median \pm interquartile range (IQR) or n (%) prevalence, as appropriate.

^aLeukocyte 8-OHdG levels are shown as median \pm IQR (Mann-Whitney U -test).

HbA_{1c}, glycosylated haemoglobin; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

NS, no statistically significant between-group differences ($P > 0.05$, Student's t -test).

compared using Student's *t*-test. Not normally distributed continuous data are presented as median \pm interquartile range (IQR), and were compared using the Mann–Whitney *U*-test. Categorical variables are presented as proportions and were compared using Fisher's exact test or χ^2 -test, as appropriate. Multivariate logistic regression was used to analyse independent factors associated with

microangiopathy in patients with type 2 diabetes. Variables with *P*-values < 0.05 by univariate analyses were included in the multivariate model. *P*-values < 0.05 were considered statistically significant.

A *post-hoc* power analysis was performed. For comparison of 8-OHdG levels between controls and patients with type 2 diabetes, the power was 100% ($n = 65$ and

Table 2. Demographic and clinical characteristics of Chinese patients with type 2 diabetes with or without microangiopathy.

Variable	Type 2 diabetes subgroup		Statistical significance
	Without microangiopathy <i>n</i> = 52	With microangiopathy <i>n</i> = 56	
Age, years	59 \pm 8	61 \pm 11	NS
Sex, male	32 (61.5)	38 (67.9)	NS
Duration of diabetes, months	80.93 \pm 59.90	126.04 \pm 84.45	<i>P</i> = 0.033
Fasting plasma glucose, mmol/l	10.2 \pm 2.3	11.8 \pm 3.7	NS
HbA _{1c} , %	8.9 \pm 2.8	9.4 \pm 3.1	NS
BMI, kg/m ²	23.8 \pm 4.6	23.4 \pm 5.8	NS
SBP, mmHg	125 \pm 38	132 \pm 35	NS
DBP, mmHg	78 \pm 24	82 \pm 22	NS
Total cholesterol, mmol/l	4.66 \pm 1.04	4.77 \pm 0.99	NS
Triglycerides, mmol/l	1.58 \pm 1.58	1.59 \pm 0.53	NS
LDL cholesterol, mmol/l	2.53 \pm 0.84	3.09 \pm 0.74	<i>P</i> = 0.042
HDL cholesterol, mmol/l	1.24 \pm 0.21	1.17 \pm 0.21	NS
Serum creatinine, mg/dl	0.79 \pm 0.23	1.12 \pm 0.38	<i>P</i> = 0.032
Albuminuria, mg/24 h	11.72 \pm 4.53	234.85 \pm 27.2	<i>P</i> = 0.005
8-OHdG, ng/ml	2.71 \pm 2.26	3.34 \pm 1.87	<i>P</i> = 0.044 ^a
Smoker	13 (25.0)	22 (39.3)	<i>P</i> = 0.010
Insulin	21 (40.4)	34 (60.7)	<i>P</i> = 0.019
Metformin	38 (73.1)	33 (58.9)	NS
Sulphonylureas	26 (50.0)	22 (39.3)	NS
α -glucosidase inhibitor	16 (30.8)	20 (35.7)	NS
Ca ²⁺ channel blocker	17 (32.7)	32 (57.1)	<i>P</i> = 0.014
ACE inhibitor/ARB	28 (53.8)	41 (73.2)	<i>P</i> = 0.035
Antiplatelet drugs (aspirin/clopidogrel)	30 (57.7)	36 (64.3)	NS

Data presented as mean \pm SD, median \pm interquartile range (IQR) or *n* (%) prevalence, as appropriate.

^aLeukocyte 8-OHdG levels are shown as median \pm IQR (Mann–Whitney *U*-test).

HbA_{1c}, glycosylated haemoglobin; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker.

NS, no statistically significant between-group differences (*P* > 0.05, Student's *t*-test).

$n = 108$, respectively; $\alpha = 5\%$). For the comparison of 8-OHdG levels between patients with type 2 diabetes with and without microangiopathy, the power was 84% ($n = 56$ and $n = 52$, respectively; $\alpha = 5\%$).

Results

Demographic and clinical characteristics

A total of 108 patients with type 2 diabetes (56 with microangiopathy, 52 without) and

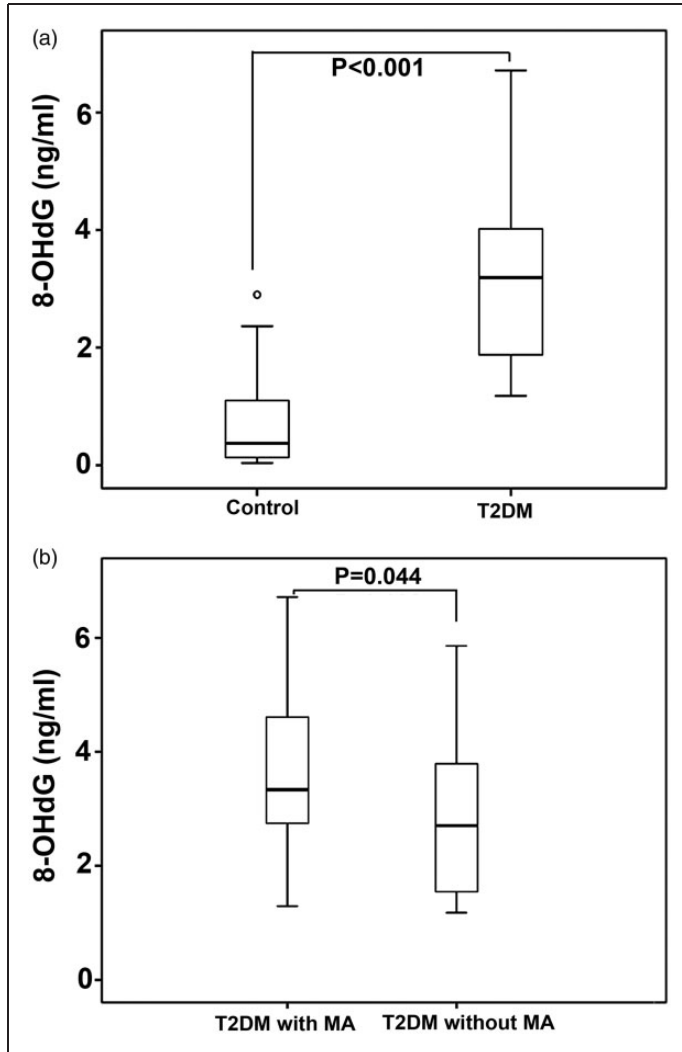


Figure 1. 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels in Chinese patients with type 2 diabetes and healthy controls. (a) 8-OHdG levels in patients with type 2 diabetes and controls; (b) 8-OHdG levels in patients with type 2 diabetes and microangiopathy and in patients with type 2 diabetes without microangiopathy. The central (heavy) black lines within the boxes represent the medians, the extremities of the boxes are the 25th and 75th percentiles, the error bars represent minimum and maximum outliers, the circle above the 'control' bar represents an extreme outlier. T2DM, type 2 diabetes; MA, microangiopathy. ($P < 0.05$, Mann-Whitney U -test).

65 healthy controls were included (Table 1). Age, sex, BMI, SBP, DBP, triglycerides, HDL-C, serum creatinine and smoking were comparable between patients with type 2 diabetes and controls (all $P > 0.05$; Table 1). Fasting plasma glucose ($P < 0.001$), HbA_{1c} ($P < 0.001$), total cholesterol ($P = 0.030$), LDL-C ($P = 0.012$) and albuminuria ($P < 0.001$) were higher in patients with type 2 diabetes compared with controls (Table 1).

In patients with type 2 diabetes grouped according to the presence or absence of microangiopathy, patient age, sex, fasting

plasma glucose, HbA_{1c}, BMI, SBP, DBP, total cholesterol, triglycerides, HDL-C, use of metformin, use of sulphonylureas, use of α -glycosidase inhibitor and use of antiplatelet drugs were comparable between the two groups (all $P > 0.05$; Table 2). Patients with type 2 diabetes and microangiopathy showed higher LDL-C ($P = 0.042$), serum creatinine ($P = 0.032$) and albuminuria ($P = 0.005$) levels versus patients with type 2 diabetes without microangiopathy. Patients with type 2 diabetes and microangiopathy also had a higher prevalence of smokers ($P = 0.010$), use of insulin ($P = 0.019$), use of Ca²⁺ channel

Table 3. Univariate and multivariate logistic regression analyses of the association between 8-OHdG in leukocyte DNA and microangiopathy in Chinese patients with type 2 diabetes ($n = 108$).

Variable	Statistical analysis					
	Univariate			Multivariate (adjusted for smokers)		
	OR	95%CI	Statistical significance	OR	95% CI	Statistical significance
8-OHdG	3.15	1.83, 8.19	$P < 0.001$	3.62	2.83, 8.71	$P = 0.002$
Duration of diabetes	1.17	1.03, 4.52	$P = 0.003$	1.84	1.16, 3.89	$P = 0.01$
Smoker	1.56	0.48, 3.15	NS	/	/	/
Age	1.34	0.39, 2.36	NS	/	/	/
BMI	2.76	0.81, 11.46	NS	/	/	/
Fasting plasma glucose	2.27	0.91, 5.74	NS	/	/	/
HbA _{1c}	1.01	0.38, 2.66	NS	/	/	/
Serum creatinine	2.32	0.78, 6.88	NS	/	/	/
Albuminuria	4.75	1.48, 15.35	$P = 0.009$	5.02	3.99, 8.08	$P < 0.001$
Triglycerides	1.16	0.34, 2.08	NS	/	/	/
Total cholesterol	2.28	0.91, 5.74	NS	/	/	/
LDL cholesterol	3.36	0.79, 14.37	NS	/	/	/
Insulin	0.99	0.91, 3.07	$P = 0.028$	0.58	0.38, 3.29	$P = 0.04$
Metformin	0.03	0.01, 1.25	NS	/	/	/
Sulphonylureas	1.48	0.91, 5.75	NS	/	/	/
α -glycosidase inhibitor	1.52	0.99, 9.63	NS	/	/	/
Ca ²⁺ channel blocker	1.03	0.07, 1.15	NS	/	/	/
ACE inhibitor/ARB	0.79	0.37, 0.95	$P = 0.01$	0.57	0.36, 0.98	$P = 0.03$
Antiplatelet drugs (aspirin/clopidogrel)	0.78	0.32, 8.93	NS	/	/	/

OR, odds ratio; CI, confidence interval; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; BMI, body mass index; HbA_{1c}, glycosylated haemoglobin; LDL, low-density lipoprotein; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker. NS, no statistically significant correlation ($P > 0.05$).

blockers ($P=0.014$), use of angiotensin-converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARBs) ($P=0.035$) and longer duration of diabetes ($P=0.033$; Table 2).

Leukocyte DNA damage detectable by 8-OHdG

Patients with type 2 diabetes were found to have higher leukocyte levels of 8-OHdG compared with controls (median \pm IQR, 3.19 ± 2.17 versus 0.38 ± 1.00 ng/ml, $P < 0.001$; Table 1 and Figure 1a). Leukocyte levels of 8-OHdG were higher in patients with type 2 diabetes and microangiopathy versus patients with type 2 diabetes without microangiopathy (median \pm IQR, 3.34 ± 1.87 versus 2.71 ± 2.26 ng/ml, $P = 0.044$; Table 2 and Figure 1b).

Univariate analyses

Univariate analyses revealed that leukocyte 8-OHdG ($P < 0.001$), duration of diabetes ($P = 0.003$), albuminuria ($P = 0.009$), insulin use ($P = 0.028$) and ACE inhibitor/ARB use ($P = 0.01$) were associated with microangiopathy in patients with type 2 diabetes (Table 3).

Multivariate analyses

Multivariate analyses revealed that leukocyte 8-OHdG levels ($P = 0.002$), duration of type 2 diabetes ($P = 0.01$), albuminuria ($P < 0.001$), use of insulin ($P = 0.04$) and use of ACE inhibitors/ARBs ($P = 0.03$) were independently associated with microangiopathy in patients with type 2 diabetes after adjustment for smoking (Table 3).

Discussion

Leukocyte 8-OHdG levels were higher in patients with type 2 diabetes compared with healthy controls. Patients with type 2 diabetes and microangiopathy were found to

have higher leukocyte 8-OHdG, serum creatinine and urine albumin levels versus patients with type 2 diabetes without microangiopathy. Multivariate analyses revealed that leukocyte 8-OHdG levels, duration of type 2 diabetes, albuminuria, use of insulin and use of ACE inhibitors/ARBs were independently associated with microangiopathy in patients with type 2 diabetes after adjustment for smoking.

The results of the present study are consistent with those already obtained in patients with type 1 diabetes²¹ and patients with type 2 diabetes.¹³ In addition, a study in patients with Leber's hereditary optic neuropathy revealed increased levels of leukocyte 8-OHdG compared with controls, due to increased oxidative stress.²² Although some evidence suggests an association between hyperglycaemia and DNA damage, few clinical studies have been performed to examine their relationship in humans with diabetes. In hyperglycaemic states, increased DNA damage might be due to impaired DNA repair following augmentation of poly(ADP) ribosylation and NF- κ B,²³ as well as impaired reduction-oxidation balance²⁴ and increased NADPH oxidase expression coupled with decreased HO-1 expression.²⁵ In the present study, increased DNA damage in leukocytes of patients with type 2 diabetes compared with control subjects, as well as increased leukocyte DNA damage in patients with type 2 diabetes and microangiopathies versus patients with type 2 diabetes without microangiopathies, emphasizes the role of oxidative stress in the development and progression of microvascular complications.

Urinary 8-OHdG has been reported to be a biomarker of oxidative DNA damage in patients with diabetic nephropathy^{26,27} and higher serum 8-OHdG levels have been observed in patients with diabetes and advanced microvascular complications.¹³ Increased 8-OHdG levels have been reported in the eyes of patients with

retinopathy²⁸ and in the serum of patients with psoriasis;²⁹ both conditions are associated with increased oxidative stress. Results of the present study concur with these published studies and suggest that 8-OHdG levels could be a predictor of diabetic microvascular complications.

Negative correlations between markers of oxidative stress and levels of antioxidant vitamins C and E have been reported, further supporting the role of oxidative stress in microangiopathy in patients with type 2 diabetes.³⁰ In addition, using a vitamin E-coated membrane during haemodialysis has been shown to reduce leukocyte 8-OHdG levels.³¹ These studies suggest that the use of antioxidant therapy might help prevent and/or alleviate microangiopathy in patients with type 2 diabetes.

In the present study, oxidative DNA damage was significantly correlated to albuminuria, but negatively correlated to serum creatinine; this suggests that albuminuria may be a more reliable and early predictor of renal dysfunction than serum creatinine, which is supported by a previous study.³² Levels of oxidative DNA damage have been reported to be significantly higher in patients with creatinine clearance <70 ml/min versus those with creatinine clearance >70 ml/min.²³ Further studies are required to clarify the causative mechanism of DNA damage in diabetic microvascular complications.

Angiotensin II is known to be involved in oxidative stress,^{33,34} and previous studies have shown that ACE inhibitors/ARBs decrease the oxidative stress in different organs.³⁵⁻³⁷ Consistent with these published studies, use of ACE inhibitors/ARBs in the present study was associated with a decreased risk of developing microangiopathy in patients with type 2 diabetes, and decreased oxidative stress is a likely mechanism. Intensive insulin treatment has been associated with decreased oxidative stress in patients with type 2 diabetes,³⁸ which could explain the association between insulin

treatment and levels of 8-OHdG observed in the present study.

The present study has a number of limitations. First, the sample size was relatively small and from a single population. Multicentre studies should be performed to increase the sample size and to represent the entire population. Secondly, the present results were obtained from a Chinese population, whose genetics, environmental factors and lifestyles differ from western populations. Thirdly, the proportion of smokers was different between the groups; however, multivariate analysis was adjusted for this variable and the association between 8-OHdG levels and microangiopathy remained significant. Finally, a larger number of markers of oxidative stress measured within the same study would provide a more complete picture of oxidative stress and microangiopathy in type 2 diabetes.^{39,40}

In conclusion, leukocyte oxidative DNA damage was high in patients with type 2 diabetes and microangiopathy. In patients with type 2 diabetes, microangiopathy was associated with leukocyte 8-OHdG levels, duration of type 2 diabetes, albuminuria and use of ACE inhibitors/ARBs. These results imply that treatments aiming to prevent oxidative DNA damage might lead to a reduction in microangiopathy, but further studies are necessary to clarify this. In the authors' opinions, the present results may also be applicable to other conditions with oxidative stress-associated microangiopathy.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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