

Original Article

Relationship between hOGG1 Ser326Cys gene polymorphism and coronary artery lesions in patients with diabetes mellitus

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Received August 10, 2015; Accepted October 7, 2015; Epub October 15, 2015; Published October 30, 2015

Abstract: To study the relationship between human 8-oxoguanine glycosylase (*hOGG1*) Ser326Cys gene polymorphism and coronary artery lesions in patients with diabetes mellitus, we analyzed 323 patients with diabetic mellitus, who underwent coronary angiography. Using PCR-RFLP, these patients were grouped into three genotypes: Cys/Cys (n=85), Ser/Ser (n=121), and Ser/Cys (n=117). Several clinical data, including history of diseases and biochemical indices were recorded. *hOGG1* mRNA expression and 8-hydroxy deoxyguanosine (8-OHdG) were measured by RT-PCR and ELISA, respectively. The quantities and severity of coronary artery with lesions were analyzed from coronary angiography. The Gensini and SYNTAX scores were detected by the unitary criteria. The 8-OHdG levels showed statistical difference among the three genotypes ($F=21.56$, $P<0.05$). Also, 8-OHdG in Cys/Cys genotype was higher than Ser/Ser and Ser/Cys genotype ($q=2.32$, $q=3.12$, $P<0.05$). In terms of the expression of *hOGG1* mRNA, the measure of *hOGG1*/β-actin showed significant difference among the three groups ($F=12.56$, $P<0.05$). On comparing two groups, *hOGG1*/β-actin in Cys/Cys genotype was higher than Ser/Ser and Ser/Cys genotypes ($q=2.32$, $q=3.12$, $P<0.05$). Percentage of 3-vessel lesions was high in Cys/Cys genotype and percentage of 1-vessel lesions was low in Ser/Cys genotype. Gensini and SYNTAX scores and ratio of complex lesions were significantly higher in the Cys/Cys genotype than the other two genotypes ($F_{\text{Gensini}}=47.16$, $F_{\text{SYNTAX}}=55.12$; $P<0.05$). *hOGG1* Ser326Cys polymorphism showed correlation with coronary artery lesions in patients with diabetes mellitus, and Cys/Cys genotype may have more impact on the severity of lesions.

Keywords: *hOGG1*, polymorphism, 8-hydroxy deoxyguanosine, diabetes mellitus, coronary artery lesions

Introduction

Type 2 diabetes mellitus is most often associated with coronary artery disease (CAD). This could possibly be attributed to the fact that diabetes mellitus is always involved in lipid metabolism disorder and elevated concentrations of reactive oxygen species (ROS), which in turn lead to oxidative stress, endothelial dysfunction, and cardiovascular complications. The damage is mainly caused by free radical-induced oxidation of DNA, proteins, and fats in the body. 8-hydroxydeoxyguanine (8-OHdG) is a major component and a key biomarker of the oxidative DNA damage induced by reactive free radicals [1, 2]. Furthermore, the oxidative modification of low density lipoproteins (LDL) in the

arterial wall by ROS results in the development of atherosclerosis. Studies have revealed that the pathogenesis of atherosclerosis is related to oxidative stress, which causes the oxidative modification of LDL by ROS and further leads to the progression of atherosclerotic plaques and possible rupture [3-5]. Additionally, it has been confirmed that an increase in the levels of 8-OHdG results in an increase in the mutation rate of genetic material, a decline in partial cell functions, and mutations in vascular genes, which ultimately lead to the formation and development of artery atherosclerosis.

Genomic DNA is susceptible to attack by ROS; while at the same time multiple DNA repair enzymes protect DNA against such oxidative

Relationship between gene polymorphism and coronary artery lesions in diabetes

Table 1. Primer sequences for hOGG1 mRNA detection using RT-PCR

Gene	Primer sequences	Product length (bp)
hOGG1	Forward: 5'-GGAAGGTGCTTGGGGAAT-3' Reverse: 5'-ACTGTCACTAGTCTCACCAG-3'	200
β -actin	Forward: 5'-ATCATGTTTGAGACCTTCAACA-3' Reverse: 5'-CATCTCTTGCTCGAAGTCCA-3'	348

damage [6]. Base excision repair (BER) and nucleotide excision repair are the main DNA repair pathways that can aid in reducing the level of 8-OHdG. Human 8-oxoguanine DNA glycosylase 1 (hOGG1) is a crucial enzyme of the BER pathway and catalyzes the removal of 8-OHdG [7, 8]. The *hOGG1* gene has been confirmed to participate in the process of DNA repair. Studies have shown that the decrease in *hOGG1* expression leads to the increase of 8-OHdG level in blood, thereby resulting in a decline in the ability to repair DNA. Furthermore, researchers have observed that the expression of *hOGG1* is affected by the Ser326Cys polymorphism in the hOGG1 gene [9].

However, the severity of coronary artery lesions in many patients is not always consistent with the degree of diabetes mellitus. The difference also lies in the susceptibility of patients to cardiovascular risk factors. CAD is a kind of multi genic and multi factorial disease, and plenty of evidences have indicated that the DNA damage influences the pathogenesis and severity of coronary artery lesions in a dose-dependent pattern [10-12]. We therefore, put forward a hypothesis that these differences may depend on the ability of an individual to repair vascular damages caused by oxidative stress. In addition, the present research was undertaken to study the association of *hOGG1* gene polymorphism with coronary artery lesions in patients with diabetes mellitus.

Material and methods

Study population

A total of 323 individuals, including 232 men and 91 women, with type 2 diabetes mellitus were enrolled in this study from September 2013 to May 2014 at the Jiangxi provincial people's hospital, Jiangxi, China. All the subjects underwent coronary angiography. The mean age of the participants at entry was 61.3 \pm 9.7 years. Patients, with age less than 30 years or

older than 75 years; with other diseases, such as cardiac valve diseases, cancer, acute or chronic inflammation, kidney and liver diseases, autoimmune diseases, cardiomyopathy, and severe heart failure; who recently underwent antioxidant treatments or a big trauma or major surgeries, were excluded from this study.

Basic clinical data of the subjects, such as age, gender, weight, blood pressure, and body mass index (BMI), along with their history of smoking and drinking alcohol were recorded. Also, the history of diseases, such as essential hypertension, diabetes mellitus, hyperlipidemia, were enquired. Smoking, drinking alcohol, and a high fat diet, were prohibited 48 h before blood sampling. 6 mL blood from each individual was taken from elbow veins 12 h after fasting; the samples were then centrifuged (3000 r/min) for 20 min and serum was obtained. Biochemical indices, including total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and fasting plasma glucose (FBG) were tested using an automatic biochemical analyzer (Hitachi, Japan).

Methods

hOGG1 gene amplification and genotyping

Genotyping was performed on leukocyte DNA that was isolated from the peripheral blood samples using blood genomic DNA extraction kit according to the manufacturer's protocol (Beijing Tianwei Era Technology Ltd., Beijing, China). The purity and quantity of the extracted DNA were assessed by measuring the OD values using an ultraviolet spectrophotometer.

2 mL blood was drawn from peripheral veins into tubes containing the anticoagulant (EDTA), and the tubes were stored at -20°C. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect the *hOGG1* gene polymorphisms. The PCR primers employed were 5'-GGAAGGTGCTTGGGGAAT-3' (forward) and 5'-ACTGTCACTAGTCTCACCAG-3' (reverse) (Table 1), and were synthesized at Takara Biotechnology Co. Ltd. (Dalian, China). Each PCR was performed in a total volume of 25 μ l in MJ PTC-220 PCR Analyzer (M. J. Research Inc. USA). The thermal cycling condi-

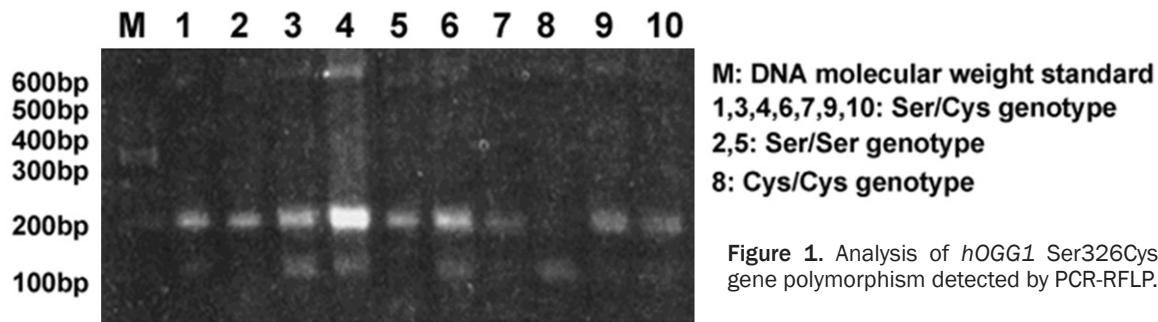


Figure 1. Analysis of *hOGG1* Ser326Cys gene polymorphism detected by PCR-RFLP.

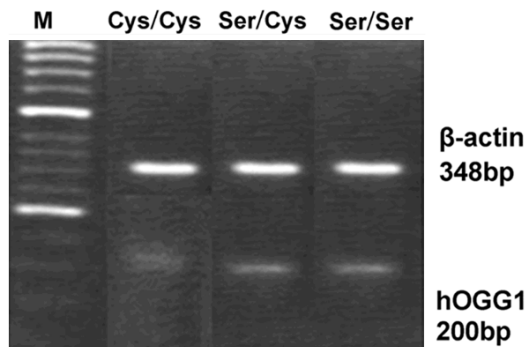


Figure 2. Expression of *hOGG1* mRNA detected by RT-PCR in 3 groups.

tions were as follows: an initial denaturation at 95°C for 3 min; followed by 35 cycles of denaturation at 94°C for 30 s, an annealing at 52°C for 30 s, and extension at 72°C for 30 s; and the final extension at 72°C for 5 min.

The products were digested for 12 h by the *Fnu4HI* restriction enzyme (Fermentas, Waltham, MA, USA) into two fragments and the fragments were separated on 2% agarose gel containing ethidium bromide. The three possible genotypes were defined based on the three distinct banding patterns observed through ultraviolet spectrophotometer: only 200 bp fragments were assigned to be Ser/Ser genotype, both 100 bp and 200 bp fragments were assigned to be Ser/Cys genotype, while only 100 bp fragments were assigned to be Cys/Cys genotype. The images of the representative gel were presented in Figure 1.

Detection of *hOGG1* mRNA expression in white blood cells and the level of 8-OHdG in plasma

10 mL blood were collected from the peripheral veins, and the plasma and white blood cells were isolated. The level of 8-OHdG in plasma were determined by enzyme-linked immunosor-

bent assay (ELISA). The white blood cells were used to obtain the total RNA based on the protocol described for the total RNA extraction testing kit using RNAi so Plus (R&D Systems China Co., Ltd. Shanghai, China). Real time PCR was employed to amplify the RNA sample and measure its quantity. β -actin was used as an internal control. The primers designed for PCR of the gene segment are listed in Table 1. A 25 μ l reaction mixture was prepared and centrifuged, and the following cycling conditions were employed: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 35 s, 55°C for 35 s, 72°C for 45 s; final extension at 72°C for 10 min. 5 μ l of the amplified products was used for agarose gel electrophoresis, and analyzed under ultraviolet spectrophotometer. The target gene segment was successfully amplified from *hOGG1*, and the subsequent steps of the RT-PCR were carried out. The fluorescent dye, SYBR Green was added to the RT-PCR reaction system. The relative expression levels of *hOGG1* mRNA in each group of genotypes were calculated with reference to the method described by Kenneth and Schmittgen [13]. The results were expressed as 100 times the ratio of expression of target gene to that of β -actin gene. The expression of *hOGG1* mRNA detected by RT-PCR in 3 groups were presented in Figure 2.

Assessment of the degree of coronary artery stenosis

The coronary artery angiography films were reviewed by two experienced cardiologists blind to the patients' clinical characteristics. A third reviewer blinded to the readings of the first two reviewers served as the arbitrator of the differences, if needed. Coronary artery stenosis was determined by quantitative coronary angiography. The lesions were defined as coronary diameter stenosis more than 50%. The record-

Relationship between gene polymorphism and coronary artery lesions in diabetes

Table 2. Comparison of clinical characteristics among three groups

Index	Ser/Cys (n=117)	Ser/Ser (n=121)	Cys/Cys (n=85)	P
Sex [male/female]	85/32	86/35	61/24	0.09
Age (years)	60.6±10.3	61.5±11.3	60.3±10.6	0.31
Diabetes mellitus duration (months)	72.6±15.7	68.9±14.7	71.2±15.1	0.26
Complications [n (%)]				
Hypertension	45 (38.8)	50 (41.5)	32 (37.9)	0.84
Hyperglycemia	22 (18.8)*	33 (27.3)*	15 (17.6)*	0.04
Stroke	16 (13.7)	18 (14.9)	11 (12.9)	0.96
Family history [n (%)]				
Coronary artery disease	21 (17.9)	25 (20.7)	15 (17.6)	0.21
Hypertension	35 (29.9)	41 (33.9)	25 (29.4)	0.73
Stroke	12 (10.3)	15 (12.4)	9 (10.6)	0.85
Risk factors				
Cigarette smoking [n (%)]	26 (22.2)*	30 (24.8)*	18 (21.2)*	0.04
Alcohol drinking [n (%)]	51 (43.6)	62 (51.2)	45 (52.9)	0.34
Body mass index (kg/m ²)	22.9±7.3	22.4±8.3	23.1±7.3	0.12
Systolic pressure (mmHg)	146.8±13.5	143.5±12.9	145.6±12.6	0.07
Diastolic pressure (mmHg)	76.6±13.1	73.5±19.3	75.3±16.9	0.25
Total cholesterol (mmol/L)	5.29±1.63*	5.64±1.73*	5.11±1.95*	0.03
Triglyceride (mmol/L)	2.14±0.54*	2.57±0.92*	2.71±0.73*	0.04
LDL-C (mmol/L)	3.19±1.73	3.64±1.75	3.11±1.90*	0.02
HDL-C (mmol/L)	1.09±1.55*	1.13±1.43*	1.15±1.95*	0.02
FBG (mmol/L)	5.87±1.23	5.64±1.16	5.75±1.27	0.21
2 h PBG (mmol/L)	9.02±1.21*	9.67±1.73*	9.09±1.15*	0.03
eGFR (mL.min ⁻¹ .1.73m ²)	74.7±17.4	71.7±18.9	75.5±13.9	0.17

LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, FBG: Fasting blood glucose, 2 h PBG: 2 hours postprandial blood glucose. *P<0.05.

Table 3. Comparison of expression levels of 8-OHdG and hOGG1 mRNA in three groups

Genotype	n	8-OHdG	hOGG1/β-actin
Ser/Cys	117	5.49±1.53	44.7±17.4
Ser/Ser	121	5.54±1.95	45.7±18.9
Cys/Cys	85	6.71±1.73 [#]	35.5±13.9 [#]
F value		21.56	12.56
p		0.02	0.01

[#]P<0.05 vs others genotypes.

ed data included the location of lesions, quantities of the coronary arteries with lesions, and the percentage of diameter stenosis of the diseased vessels. The methods have been described below.

Quantities of coronary arteries with lesions: If the diameter and degree of stenosis of main coronary artery lesions was beyond 50%, the coronary artery was considered as coronary artery with lesions. All the patients were divid-

ed into 1-, 2-, and 3-vessel lesions according to the quantities of coronary artery with lesions, while the left main coronary artery (LMCA) was considered as 2-vessel lesions.

Types of coronary artery lesions: Coronary artery lesions were divided into A-, B (B₁/B₂-), and C-type lesions according to ACC/AHA standards. If several kinds of lesions existed simultaneously in one patient's coronary artery, the coronary artery lesions were defined as more complex type. We observed that the A- and B₁-type lesions belonged to the category of simple lesions, while the B₂-/C-types were described as complex lesions.

Degree of complexity of coronary artery lesions: The degree of complexity of coronary artery lesions was evaluated using Gensini and SYNTAX scoring system. The Gensini score was calculated according to the Gensini score criteria of the Coronary Artery Surgery Study (CASS):1 point for stenosis degree <25%, 2

Relationship between gene polymorphism and coronary artery lesions in diabetes

Table 4. Multiple logistic regression analysis between clinical factors and 8-OHdG in three groups

Clinical factors	β	Wald	SE	OR (95% CI)	P
Hyperglycemia	0.784	6.350	1.907	2.796 (1.18~4.77)	0.008
Cigarette smoking	0.698	5.775	1.652	2.921 (1.79~3.98)	0.013
Total cholesterol	1.976	9.781	2.119	4.835 (2.438~11.914)	0.002
Triglyceride	1.356	8.528	2.009	3.976 (1.794~7.706)	0.005
2 h PBG	1.755	9.221	2.905	3.001 (1.883~6.772)	0.009
LDL-C	1.179	6.901	2.018	3.736 (2.001~4.413)	0.000
HDL-C	-1.126	4.997	2.338	1.551 (1.076~2.163)	0.000

Table 5. Association between *hOGG1* gene polymorphism and quantities of coronary artery with stenosis

Genotype	n	Quantities of coronary artery with stenosis (n, %)		
		1-vessel	2-vessel	3-vessel
Ser/Cys	117	42 (35.9)	40 (34.2)	35 (29.9)
Ser/Ser	121	43 (35.5)	41 (33.9)	37 (30.6)
Cys/Cys	85	21 (24.7)	32 (37.6)	32 (37.6)
χ^2		3.08	2.79	1.58
P value		0.215	0.332	0.454

points for 25%~50%, 4 points for 50%~75%, 8 points for 75%~90%, 16 points for 90%~99%, and 32 points for 100% (total conclusion). The scores of different parts of lesions were modified with corresponding coefficients based on the Gensini standard. For example, LMCA $\times 5$; Proximal left anterior descending (LAD. Pro.) $\times 2.5$; middle left anterior descending (LAD. Mid.) $\times 1.5$; Distal left anterior descending (LAD. Dis.) $\times 1$; First diagonal branch (DIA 1st) $\times 1$; second diagonal branch (DIA 2nd) $\times 0.5$; Proximal left circumflex (LCX Pro.) $\times 2.5$; [middle left circumflex (LCX. Mid.), Posterior descending artery (PDA), and obtuse marginal artery (OMA)] $\times 1$, rear side artery $\times 0.5$; [Proximal, middle, and distal right coronary artery (RCA)] $\times 1$. The Gensini score for each patient was the total of all the branch points. The SYNTAX score was calculated according to the features of lesions, such as total occlusion lesions, bifurcation lesions, lesions with length >20 mm, calcification lesions, thrombosis lesions, and disseminated/small vessel lesions (www.coronary-score.com).

Statistical analysis

All data were analyzed using the SPSS13.0 software. Quantitative data were expressed by mean \pm SD. Analysis of variance was used for

the comparison of quantitative data among the three groups. If the quantitative data of the three genotypes were statistically significant, further comparison between two groups was carried out with the S-N-K test. Count data were expressed as percentages and χ^2 test was applied. P value <0.05 was considered to be statistically significant.

Results

Basic characteristics of the study participants

All participants were categorized into three genotypes according to the characters of banding patterns detected by PCR-RFLP. They were respectively Cys/Cys (n=85), Ser/Cys (n=117), and Ser/Ser (n=121). Baseline clinical data of all patients are shown in **Table 2**. No significant differences were observed among the three genotypes in terms of gender (P=0.07), age (P=0.17), BMI (P=0.09), diabetes mellitus duration (P=0.11), family history (P=0.07), FBG (P=0.07), SBP (P=0.51), and DBP (P=0.32). However, percentage of hyperlipemia, percentage of cigarette smoking, total cholesterol, triglycerides, HDL-C, LDL-C, and 2 h-PBG showed statistical differences (P<0.05) among the 3 groups.

Comparison of levels of 8-OHdG and *hOGG1* mRNA expression among three groups

Table 3 presents the levels of 8-OHdG in three groups (F=21.56, P<0.05). Compared between two groups each other, the levels of 8-OHdG in Cys/Cys genotype was higher than others genotypes (q=4.56, q=5.12, P<0.05). However, no statistical difference was observed between Ser/Ser and Ser/Cys genotypes (q=3.06, P>0.05).

The statistical difference in terms of *hOGG1* mRNA expression was also observed in three groups (F=12.56, P<0.05). Compared between two groups each other, Cys/Cys genotype showed lower expression of *hOGG1* mRNA than others genotypes (q=2.32, q=3.12, P<0.05), while no significant difference was observed between Ser/Ser genotype and Ser/Cys genotype (q=1.26, P>0.05).

Relationship between gene polymorphism and coronary artery lesions in diabetes

Table 6. Relationship between *hOGG1* gene polymorphism and types of coronary artery lesions

Genotype	n	Simple lesions			Complex lesions		
		A type	B ₁ type	Total	B ₂ type	C type	Total
Ser/Cys	117	21 (17.9)	34 (29.1)	55 (47.0)	21 (17.9)	41 (35.0)	62 (54.9)
Ser/Ser	121	12 (9.9)	35 (28.9)	47 (38.8)	31 (25.6)	43 (35.5)	74 (61.1)
Cys/Cys	85	10 (11.8)	13 (15.3)	23 (27.1) [#]	22 (25.9)	40 (47.1)	62 (73.0) [#]

[#]P<0.05 vs others genotypes.

Table 7. Relationship between *hOGG1* gene polymorphism and the Gensini and SYNTAX scores

Genotype	n	Gensini score	SYNTAX score
Ser/Cys	117	21.8±10.7	29.8±15.7
Ser/Ser	121	39.8±12.7	33.8±10.5
Cys/Cys	85	48.7±15.3 [#]	39.5±17.2 [#]
F value		47.16	55.12
P		0.02	0.02

[#]P<0.05 vs others genotypes.

Association between clinical factors and levels of 8-OHdG

While **Table 2** presented the statistical differences among the three groups in terms of the clinical factors, **Table 4** shows the results of multivariate logistic regression analysis after adjusting for clinical variables, such as hyperlipemia history, cigarette smoking, total cholesterol, triglycerides, HDL-C, LDL-C and 2hPBG, which revealed that these clinical factors were positively correlated with the levels of 8-OHdG.

Association between *hOGG1* gene polymorphism and quantities of coronary artery with stenosis

The percentage of 3-vessel lesions in Cys/Cys genotype was 37.6%, and the percentage was 29.9% and 30.6% respectively in Ser/Cys genotype and Ser/Ser genotype. The percentage of 1-vessel lesions in Cys/Cys genotype was 24.7%, and it was 35.9% and 35.5% respectively in Ser/Cys genotype and Ser/Ser genotype. However, the percentage of quantities of coronary arteries with stenosis is showed no significant differences (P>0.05) (**Table 5**).

Association between *hOGG1* gene polymorphism and types of coronary artery lesions

In this study, simple lesions included A and B₁ type lesions, and complex lesions included B₂

and C type lesions. Compared among the three genotypes, the study revealed that the percentage of simple lesions in Cys/Cys genotype was less than others genotypes and the percentage of complex lesions in Cys/Cys genotype was more than others genotypes (P<0.05) (**Table 6**).

Association between *hOGG1* gene polymorphism and the Gensini score or SYNTAX score

The Gensini score and SYNTAX score in Cys/Cys genotype was 48.7±15.3 points and 39.5±17.2 points respectively, the score in Ser/Cys genotype was 21.8±10.7 points and 29.8±15.7 points respectively, and the score in Ser/Ser genotype was 39.8±12.7 points and 33.8±10.5 points respectively. All statistical data were listed in **Table 7**. The differences of Gensini score and SYNTAX score among three groups were statistically significant ($F_{\text{Gensini}}=47.16$, P<0.05; $F_{\text{SYNTAX}}=55.12$, P<0.05). Compared between two genotypes each other, the scores of Cys/Cys genotype were higher than others genotypes and the difference was statistically significant ($q_{\text{Gensini}}=5.25$, $q_{\text{SYNTAX}}=6.42$, P<0.05). On the contrary, the scores did not reveal statistical significant difference between Ser/Ser genotype and Ser/Cys genotype ($q_{\text{Gensini}}=1.25$ and $q_{\text{SYNTAX}}=2.02$, P>0.05) (**Table 7**).

Discussion

Currently, much attention has been focused on the Ser326Cys polymorphism in the *hOGG1* gene sequences and in exploring the correlation between this polymorphism and susceptibility to cancer. Many researchers believe that the activity of *hOGG1* depends on the *hOGG1* gene polymorphisms, and that *hOGG1* Cys326 is a weaker polymorphism as compared to *hOGG1* Ser326 in the ability to repair DNA damage. The DNA in human cells is prone to oxidative damage by various endogenous biochemical processes; nevertheless, exposure to hazardous environmental chemicals and lifestyle

factors are important determinants of the extent of oxidative DNA damage [14]. Metabolic syndrome is defined by the presence of a number of risk factors for cardiovascular diseases, including glucose intolerance, hypertension, hyperlipidemia, insulin resistance, and obesity [15]. Active oxygen free radicals attack the eighth carbon atom in guanine of DNA molecules and leads to the production of an oxidative adduct: 8-OHdG, a modification product of oxidative damaged DNA, which can potentially lead to the DNA mutation and induce carcinogenesis. hOGG1 is a DNA repair enzyme; it can specifically remove 8-OHdG and repair damaged DNA. As the representative of repair enzymes, studies have confirmed that the expression of *hOGG1* gene polymorphisms may affect the activity of hOGG1 [16]. In 2000, Hananka et al proposed utilizing the expression of hOGG1 mRNA in peripheral blood lymphocytes as a marker for oxidative DNA damage [17].

Many studies have confirmed the association of atherosclerosis with elevated levels of oxidative stress in the body. In diabetes mellitus patients, there are many factors that cause an imbalance between formation and clearance of reactive oxygen species in the body and result in oxidative stress injuries. For instance, a study by Martinez-Hervas et al has shown that hyperlipidemia is associated with elevated levels of oxidative DNA damage in peripheral blood mononuclear cells (PBMCs) [18]. Furthermore, patients with type 2 diabetes mellitus or vascular disease have been shown to contain higher levels of 8-oxo-7,8-dihydro-2'-deoxyguanosine 8-oxodG in lymphocytes as compared to healthy subjects [19, 20]. Certain *in vitro* studies have revealed that high glucose induces a significant increase in the intracellular ROS in human umbilical vein endothelial cells, along with a significant rise in the NADPH oxidase activity, the expression levels of mRNA, and levels of Nox4 and Nox2/gp91phox proteins, which form part of the NADPH oxidase subunit. Researchers also found that the body's antioxidant system gets weakened in individuals with hyperglycemia and results in an increase in oxidative stress. Furthermore, the fluctuation in blood sugar levels provides a stimulus to cause a more severe oxidative stress reaction. Meanwhile, hyperglycemia concomitant with the disorder of lipid metabolism can result in an increase in the production of reactive oxygen

species and oxidative stress. Oxidative modification of LDL is induced by the rise in reactive oxygen species, thereby forming ox-LDL, ox-LDL macrophage foam cell formation, which in turn causes atherosclerotic plaque formation. Therefore, high glucose-induced mitochondrial ROS, excessive production of free radicals, decreased antioxidant systems, and increased oxidative stress form part of an important pathophysiologic mechanism and have become the central events in the development of diabetic complications. The linkage between high blood glucose levels and oxidative damage to DNA in lymphocytes is well established in diabetics [19]. In addition, an association between the glycemic status and oxidative damaged DNA in PBMCs has been described in well-controlled diabetes patients [21].

In this study, the clinical factors, including hyperlipemia history, cigarette smoking, total cholesterol, triglycerides, HDL-C, LDL-C and 2 h PBG, were found to be positively correlated with the levels of 8-OHdG by logistic multivariate regression analysis (**Table 4**). This finding confirmed that metabolic risk factors are associated with elevated levels of oxidative damaged DNA in PBMCs. Another study by Espinosa et al showed higher levels of 8-oxodG in leukocytes in patients with essential hypertension than subjects with normal blood pressure at the same age and body weight, although their statistical analysis did not control for metabolic or lifestyle factors [22].

The level of 8-OHdG was found to increase significantly in our study. Although the current analysis resulted in no clear association between *hOGG1* Ser326Cys gene polymorphism and quantities of coronary artery with stenosis in patients with diabetes, the complexity of coronary artery lesions in patients with Cys/Cys genotype was observed to be more severe. The Gensini score and the SYNTAX score, which reflect the severity of coronary artery lesions (such as total occlusion lesions, bifurcation lesions, calcification lesions, thrombosis lesions, and diffuse lesions), were higher in patients with Cys/Cys genotype than in Ser/Cys genotype and Ser/Ser genotype.

The overall differences observed among the three genotypes maybe attributed to fact that hOGG1 is a specific 8-OHdG excision repair enzyme, and that mutation, deletion, or poly-

morphisms in the *hOGG1* gene lead to the reduction or loss of the cell's ability to repair 8-OHdG. Moreover, a correlation between the *hOGG1* Ser326Cys polymorphism and the amount of serum 8-OHdG was observed in the patients (Table 2). These findings were, therefore, in agreement with the lower levels of *hOGG1* expression found in the Cys/Cys genotype than in the Ser/Ser genotype and Ser/Cys genotype; in contrast, the level of 8-OHdG in the Cys/Cys genotype was correspondingly higher than the Ser/Ser and Ser/Cys genotypes. Furthermore, it was confirmed that the *hOGG1* gene polymorphisms affect the expression of *hOGG1*, thereby decreasing its ability to recognize and remove 8-OHdG, lowering its ability to repair DNA damage in double stranded DNA, and consequently promoting the initiation and progression of atherosclerosis on the premises of risk factors, such as blood sugar, blood lipid, and other collaborative participation. Our data corroborated the observations made by Wang et al, that patients who carry the Cys/Cys alleles display poor sensitivity to insulin and are more susceptible to coronary artery diseases [23].

However, there were several shortcomings in this study. Firstly, genetic Hardy-Weinberg testing was not applied, therefore it was not known whether the population included in the study met the H-W balance, and if the sample was a good representative of the three groups under consideration. Secondly, the study was not a randomized, double blind study. Finally, increasing the number of individuals may have increased the statistical power of the study.

In conclusion, the *hOGG1* Ser326Cys gene polymorphism was found to be correlated with the coronary artery lesions in patients with diabetes mellitus, and the Cys/Cys genotype was indicated to have a greater effect on the severity of coronary artery lesions. Nevertheless, an evidence-based study with a larger sample size is further required to validate the findings of the current study.

Disclosure of conflict of interest

None.

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Relationship between gene polymorphism and coronary artery lesions in diabetes

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