The role of oxidative DNA damage and GSTM1, GSTT1, and hOGG1 gene polymorphisms in coronary artery disease risk

Ela Kadıoğlu, Gülten Taçoy, Eren Özçağlı[1], Kaan Okyay[2], Mehmet K. Akboğa[3], Atiye Çengel*, Semra Şardaş[4]*

Departments of Toxicology Faculty of Pharmacy and *Cardiology Faculty of Medicine, Gazi University; Ankara-*Turkey*
[1]Department of Toxicology, Faculty of Pharmacy, İstanbul University; İstanbul-*Turkey*
[2]Department of Cardiology, Medical School, Başkent University; Ankara-*Turkey*
[3]Department of Cardiology, Türkiye Yüksek İhtisas Training and Research Hospital; Ankara-*Turkey*
[4]Department of Toxicology, Faculty of Pharmacy, Marmara University; İstanbul-*Turkey*

Abstract

Objective: Atherosclerotic coronary artery disease (CAD) appears to be a multifactorial process caused by the interaction of environmental risk factors with multiple predisposing genes. Therefore, in this study we aimed to determine the role of oxidative DNA damage and some variations in glutathione S-transferase (GSTM1 and GSTT1) and DNA repair (hOGG1) genes in CAD risk.

Methods: A case-control study was conducted on 59 individuals who had undergone coronary angiographic evaluation. Of these, 29 were patients diagnosed with CAD (mean age =61.5±10.3) and 30 were controls examined for reasons other than suspected CAD and who had angiographically documented normal coronary arteries (mean age =60.4±11.6). Basal DNA damage as well as pyrimidine and purine base damage were evaluated in peripheral blood lymphocytes using the modified comet assay. Polymerase chain reaction-restriction length polymorphism (PCR-RFLP)-based assay was used for genotyping.

Results: Basal DNA damage levels in patients [9.16 (3.26)] were significantly higher than those in controls [7.59 (3.23); p=0.017], and basal DNA and pyrimidine base damage levels were significantly correlated with disease severity based on Gensini scoring (r=0.352, p=0.006; r=0.318, p=0.014, respectively). However, no significant differences were observed in terms of oxidized DNA bases between patients and controls. The frequencies of studied genotypes (GSTM1, GSTT1, and hOGG1) were similar between groups.

Conclusion: The results of this study pointed out the role of DNA damage in CAD and its severity. However, GSTM1, GSTT1, and hOGG1 gene polymorphisms seemed to have no effect on individual susceptibility for disease progression. *(Anatol J Cardiol 2016; 16: 931-8)*

Keywords: coronary artery disease, DNA damage, GSTM1, GSTT1, hOGG1

Introduction

Coronary artery disease (CAD), which is one of the most frequent causes of death, is associated with chronic inflammation of the arterial intima and is characterized by the formation of atherosclerotic plaques (1). Epidemiological studies have demonstrated that CAD is a multifactorial disease caused by one or more genes in combination with environmental factors, including gene–environment interactions (2). Oxidative DNA damage has been investigated in CAD cases, and some evidence of increased reactive oxygen species (ROS) formation and impaired antioxidant protection during disease progression has been provided (3–6). Moreover, some genetic variants have been associated with the risk of developing CAD (7, 8). Therefore, a study of the interactions between the polymorphisms of several genes, especially the ones coding phase I and phase II detoxification enzymes such as CYPs or glutathione S-transferase (GSTs), and oxidative DNA damage may be helpful in understanding the mechanism of this atherosclerotic disease. The GST gene family, which encodes phase II metabolizing enzymes, plays an important role in the biotransformation and detoxification of different xenobiotics and endogenous compounds. Since many GST genes are polymorphic, there has been considerable interest in determining whether particular allelic variants are associated with an altered risk (or outcome) of a variety of diseases, including CAD (9, 10).

Two polymorphic genes of this family, GSTM1 and GSTT1, present null alleles that consequently do not produce the res-

Address for correspondence: Dr. Ela Kadıoğlu, Gazi Üniversitesi Tıp Fakültesi
Eczacılık Fakültesi Toksikoloji Bölümü, 06330 Ankara-*Türkiye*
Phone: +90 312 202 30 83 Fax: +90 312 222 23 26 E-mail: ela1015@hotmail.com; ela@gazi.edu.tr
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pective enzyme when the genotype is homozygous. The polymorphisms of these two enzymes are often studied together for many diseases. These enzymes convert many electrophilic toxic chemicals, which damage macromolecules including DNA, to less harmful compounds through glutathione conjugation. Many carcinogenic and toxic molecules that produce DNA damage can contribute to the development of CAD. The polymorphisms of GSTM1 and GSTT1 genes may affect enzymatic activity and susceptibility to CAD. On the other hand, the polymorphisms of DNA repair genes may also be associated with insufficient DNA repair and may influence individual susceptibility to CAD. Base excision repair (BER) is a DNA repair pathway that operates on small lesions such as oxidized or reduced bases, fragmented or non-bulky adducts, or those produced by methylating agents (11). hOGG1 is a DNA glycosylase that recognizes and removes the altered base (8-OHdG) in the BER pathway. It is encoded by the hOGG1 gene localized on chromosome 3p25. Among several polymorphisms of the hOGG1 gene, Ser326Cys polymorphism is one of the most important, in which C/G polymorphism at position 1245 in exon 7 results in an amino acid substitution of serine with cysteine in codon 326 (12).

The alkaline comet assay, which detects single-strand breaks (SSBs) as well as alkaline-labile sites, is one of the most promising biomarkers. Recently, it has been modified to analyze oxidative DNA damage using lesion-specific enzymes [(endonuclease III (Endo III) and formamidopyrimidine glycosylase (Fpg)] and has been accepted as a reliable biomarker for oxidative stress (13).

In the present study, we aimed to investigate the roles of oxidative DNA damage and some polymorphisms in oxidative stress (GSTM1 and GSTT1) and DNA repair (hOGG1 Ser326Cys) genes in terms of individual susceptibility to CAD. We also investigated the interaction between oxidative DNA damage and the polymorphisms of these genes in CAD patients.

Methods

Subjects

The study population consisted of 59 patients who had undergone coronary angiography. Of these, 29 patients had angiographically documented CAD (24 men, 5 women; mean age, 61.5±10.3 years); 30 patients with angiographically documented normal coronary arteries were considered as the control group (24 men, 6 women; mean age, 60.4±11.6 years). Significant CAD was defined as a stenosis of ≥50% in any of the main coronary arteries, and control subjects were enrolled if they had neither a history nor clinical evidence of CAD. The severity of CAD was determined by the number of affected vessels (one-, two-, or three-vessel disease). The severity of the disease was also evaluated by calculating the Gensini score (14, 15).

Details of patients and controls that were recorded at the time of sampling included age, gender, smoking status, a family history of CAD, a history of hypertension, diabetes, and hyperlipidemia, and serum lipid profiles [low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), total cholesterol, and triglyceride levels]. Exclusion criteria were the presence of neoplastic disease, acute or chronic inflammatory disease, and immunological disease and a history of recent major surgical procedures. Medications used by patients generally included calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, nitrates, statins, and oral aspirin. None of the patients or control subjects were receiving any antioxidant drugs such as vitamins. Written informed consent was obtained from all participants, and the study was approved by the local ethics committee.

Blood sampling

Lymphocytes were obtained from venous blood samples supplemented with heparin for the comet assay. Blood for genotyping was collected in tubes coated with EDTA. After collection and during transport, the samples were kept at 4°C and protected from light. The comet assay was started no later than 24 h after sampling. Blood samples for genotyping were stored at –80°C until use. Samples from patients and controls were concurrently handled.

Modified comet assay

From each individual, 2 mL of whole blood was added to 1% Mg^{2+}-free phosphate-buffered saline (PBS) and underlayed with Histopaque 1077 (Sigma, Steinheim, Germany). Cells were immediately centrifuged at 200 xg for 3 min at 4°C. Lymphocytes were retrieved from just above the boundary between PBS and Histopaque, added to PBS, and centrifuged at 200 xg for 3 min at 4°C.

The basic alkaline technique was followed, with modifications for the detection of oxidized bases using bacterial repair endonucleases, as previously described (16). Before electrophoresis, the lymphocytes were treated with either buffer to examine basal DNA damage or Endo III or Fpg to examine pyrimidine or purine base damage, respectively. Freeze-dried Endo III (specific for oxidized pyrimidines) and Fpg (recognizes altered purines, including 8-oxoGua) were kindly provided by Dr. Andrew Collins, University of Oslo, Oslo, Norway. The percentage of DNA in the tail (TD%, percentage of tail DNA) was taken as a measure of DNA break frequency. TD% was assessed in 50 cells using the Comet Assay III image analysis system (Perceptive Instruments, UK). Analysis was blindly performed by a slide reader. Subtracting the TD% without enzyme incubation (i.e., strand breaks) from the TD% with enzyme incubation gives the net amount of damage, which is represented by pyrimidine (Endo III sites) or purine base damage (Fpg sites) (13).

Genotype analysis

Genomic DNA, which was isolated from frozen peripheral blood samples using the sodium perchlorate/chloroform extraction method (17), was used as a template in PCR-based genotyping analysis. Genotypes for the selected genes were determined by the PCR/RFLP method.

GSTM1 gene

The most important polymorphism encodes a partial gene deletion at the GSTM1 locus on chromosome 1p13.3 (GSTM1 null genotype), resulting in the complete absence of GSTM1 enzyme activity. For detecting this deletion, the PCR primers used were Primer 1 (AA1-forward): 5′-CGC CAT CTT GTG CTA CAT TGC CCG-3′; Primer 2 (AA2-reverse): 5′-ATC TTC TCC TCT TCT GTC TC-3′; and Primer 3 (AA3-reverse): 5′-TTC TGG ATT GTA GCA GAT CA-3′ (Thermo, Ulm, Germany). PCR was performed in a total volume of 10 µL consisting of 100 ng of genomic DNA, 0.25 mM dNTP (Fermentas Hanover, MD, USA), 1.5 mM $MgCl^2$, 0.4 µM of each primer, and 1 unit of Taq DNA polymerase (Fermentas) in 1x PCR buffer. The PCR program consisted of a 2-min initial denaturation step at 94°C, followed by 35 cycles of 1-min denaturation at 94°C, 1 min 30 s of annealing at 53°C, and 2-min elongation at 72°C. The final elongation was for 10 min at 72°C. The specific size of the GSTM1 gene PCR product (231 bp) and a control band (158 bp) was assessed after electrophoresis on a 2% agarose gel.

GSTT1 gene

The GSTT1 gene, located on chromosome 22q11.23, exhibits a deletion polymorphism similar to that of the GSTM1 gene. GSTT1 genotyping was determined by PCR using the forward primer: 5′-AGG CAG CAG TGG GGG AGG ACC-3′ and reverse primer: 5′-CTC ACC GGA TCA TGG CCA GCA-3′. GSTP1 primers (GSTP1-forward: 5′-ACC CCA GGG CTC TAT GGG AA-3′ and GSTP1-reverse: 5′-TGA GGG CAC AAG AAG CCC CT-3′) were also included as controls in the PCR mixture to see the independent amplification of each sample. PCR was performed in a total volume of 10 µL consisting of 100 ng of genomic DNA, 0.2 mM dNTP (Fermentas Hanover, MD, USA), 2 mM $MgCl^2$, 0.25 µM of each primer, and 1 unit of Taq DNA polymerase (Fermentas) in 1x PCR buffer. The PCR program consisted of a 5-min initial denaturation step at 94°C, followed by 30 cycles of 1-min denaturation at 94°C, 1-min annealing at 60°C, and 2-min elongation at 72°C. The final elongation was for 10 min at 72°C. The specific size of the GSTT1 gene PCR product (138 bp) and a control band (158 bp) was assessed after electrophoresis on a 2% agarose gel. Null genotypes were scored after GSTP1 amplifications were confirmed.

hOGG1 gene

For the genotyping of codon 326 of hOGG1, the following primers were designed to encompass the Ser326Cys polymorphic site: hOGG1-forward 5′: 5′-ACT GTC ACT AGT CTC ACC AG-3′; hOGG1-reverse 3′: 5′-TGA ATT CGG AAG GTG CTT GGG GAA T-3′ (Thermo, Ulm, Germany). PCR was performed in a total volume of 20 µL consisting of 100 ng of genomic DNA, 0.25 mM dNTP (Fermentas Hanover, MD, USA), 1.5 mM $MgCl^2$, 0.3 pmol of each primer, and 1 unit of Taq DNA polymerase (Fermentas) in 1x PCR buffer. The PCR program consisted of a 2-min initial denaturation step at 94°C, followed by 33 cycles of 15-s denaturation at 94°C, 30-s annealing at 60°C, and 35-s elongation at 72°C. The final elongation was for 10 min at 72°C. PCR yielded a 207-bp product. After PCR, a 10-µL aliquot of the amplification product was digested (overnight at 37°C) by 2 units Fnu4HI (NEB, England, UK). The digestion products were separated after electrophoresis on a 3% NuSieve agarose gel, and the DNA fragments were visualized with 10 mg/mL ethidium bromide. Consequently, the Ser/Ser, Ser/Cys, and Cys/Cys genotypes resulted in 207-bp; 207-, 107-, and 100-bp; and 107- and 100-bp digestion products, respectively.

Statistical analysis

Data analysis was performed using SPSS for Windows, version 11.5. Whether the continuous variables were distributed normally or not, they were determined using the Shapiro–Wilk test. Data were shown as mean±standard deviation or median (IQR) for continuous variables, where appropriate. The differences in medians between the control and CAD groups were compared by the Mann–Whitney U test. Nominal data were evaluated by the continuity correction chi-square or Fisher's exact test, where applicable. Univariate logistic regression analyses were applied for determining the associations between each continuous variable and the presence of CAD. Odds ratios and 95% confidence intervals were calculated. Degrees of association between the continuous variables were evaluated by Spearman's rank correlation test. Whether each genotype (i.e. GSTM1, GSTT1 and hOGG1) has a statistically significant effect on the presence of CAD was evaluated by multiple logistic regression analyses after adjustment for age, gender, the presence of hypertension, and smoking habits. Adjusted odds ratios and 95% confidence intervals were also calculated. A p value of <0.05 was considered statistically significant.

Results

The demographic and clinical characteristics of patients and controls are presented in Table 1. A statistically significant association was found between age and the presence of CAD (p=0.02, OR=1.06 95% CI=1.01–1.12). The number of hypertensive subjects in the patient group was significantly higher than that in the control group (p=0.015, OR=4.64, 95% CI=1.48–14.54). Seven patients had a family history of CAD, whereas none of the controls had a family history of CAD (p=0.005). No significant differences were observed between the groups with respect to gender, smoking habits, and other risk factors.

The distribution and frequency of GSTM1, GSTT1, and hOGG1 genotypes for patients and controls are given in Table 2. Among the control population, the frequency of selected polymorphic variants were GSTM1 (null), 0.59; GSTT1 (null) 0.21; hOGG1 (Cys/Cys), 0.07, and these frequencies were not different from those (0.47, 0.17, and 0.07, respectively) in the studied patient population. The results of multiple logistic regression analyses after adjustment for age, gender, the presence of hypertension, and smoking habits are also given in Table 2. No deviation from the Hardy–Weinberg equilibrium was seen for any of the genotypes studied.

Significant differences were observed in basal DNA damage in terms of TD% between patients and controls (Table 3). Basal

Table 1. Demographic and clinical characteristics of patients and controls

	CAD patients (n=29)	Controls (n=30)	OR (95%CI)	*P*
Age, years±SD	61.5±10.3	54.4±11.6	1.06 (1.01–1.12)	**0.020**†
Gender, Men/Women, n (%)	24 (82.8)/5 (17.2)	24 (80)/6 (20)	0.83 (0.22–3.10)	1.000‡
Hypertension, n (%)	17 (60.7)	7 (25)	4.64 (1.48–4.54)	**0.015**‡
Diabetes mellitus, n (%)	11 (39.3)	11 (40.7)	0.94 (0.32–2.77)	1.000‡
Hyperlipidemia, n (%)	9 (32.1)	7 (25.9)	1.35 (0.42–4.36)	0.833‡
Smoking, n (%)	16 (55.2)	11 (40.7)	1.79 (0.62–5.17)	0.417‡
Family history, n (%)	7 (24.1)	0 (0)	NC	**0.005**¶
Total cholesterol, mg/dL	187.7±36.1	188.3±62.1	1.00 (0.99–1.01)	0.965†
LDL-cholesterol, mg/dL	101.7±21.3	102.8±44.5	0.99 (0.98–1.01)	0.918†
HDL- cholesterol, mg/dL	45.3±10.4	47.3±19.3	0.99 (0.95–1.03)	0.649†
Triglyceride, mg/dL	265.2±344.4	164.4±84.5	1.00 (0.99–1.01)	0.230†
Gensini score	54.1±36.8	7.4±29.9	1.06 (1.03–1.10)	**<0.001**†

‡Continuity correction chi-square test; ¶Fisher's exact test; †Univariate logistic regression analyses; CAD - coronary artery disease; HDL - high–density lipoprotein cholesterol; LDL - low-density lipoprotein cholesterol; NC - not calculated

Table 2. Distribution n (%) of GSTM1, GSTT1 and hOGG1 genotypes in patients and controls

	CAD patients	Controls	OR (95% CI)	*P*	Adjusted* OR (95% CI)	*P*
GSTM1						
Active	12 (41.4)	16 (53.3)				
Null	17 (58.6)	14 (46.7)	1.619 (0.578–4.534)	0.510†	0.325 (0.082–1.293)	0.111
GSTT1						
Active	23 (79.3)	25 (83.3)				
Null	6 (20.7)	5 (16.7)	1.304 (0.350–4.858)	0.950†	1.005 (0.200–5.041)	0.995
hOGG1						
Ser/Ser	15 (51.7)	17(56.7)				
Ser/Cys	12 (41.4)	11 (36.7)	1.236 (0.423–3.615)	0.909†	1.230 (0.327–4.631)	0.759
Cys/Cys	2 (6.9)	2 (6.7)	1.133 (0.142–9.065)	1.000‡	0.510 (0.038–6.861)	0.612

*Adjusted for age, gender, presence of hypertension and smoking habits; †Continuity correction chi-square test; ‡Fisher's exact test; CAD - coronary artery disease

DNA damage levels in patients [9.16 (3.26)] were significantly higher than those in controls [7.59 (3.23); p=0.017]. The addition of Endo III or Fpg enzymes caused an increase in SSBs in both patients and controls, demonstrating the presence of pyrimidine and purine base damage in DNA. However, these increases were not statistically different between the groups (p>0.05).

The influences of GSTM1, GSTT1, and hOGG1 genotypes on oxidative DNA damage parameters are presented in Table 4. No significant differences were observed between individuals carrying the GSTM1, GSTT1, and hOGG1 variant or the wild genotype in terms of oxidative DNA damage parameters in both controls and patients. However, basal DNA damage levels were statistically different in patients as compared with in controls carrying the same genotypes. Patients carrying the GSTT1 active genotype or Ser/Cys + Cys/Cys variants of the hOGG1 showed higher levels of basal DNA damage compared with controls carrying the same genotypes (p=0.014 and 0.022 respectively).

Table 3. Oxidative DNA damage in patients and controls. Values are median (IQR)

	CAD patients	Controls	*P*
Basal DNA damage	9.16 (3.26)	7.59 (3.23)	**0.017**
Pyrimidine base damage (Endo III sites)	15.04 (12.07)	11.02 (6.98)	0.08
PBD (Fpg sites)	12.38 (6.98)	12.24 (4.80)	0.55

Mann-Whitney U test. CAD - coronary artery disease; PBD - purine base damage

The correlations between the Gensini score as a marker of CAD severity and oxidative DNA damage parameters are presented in Table 5. There were significant correlations between the Gensini score and basal DNA damage (r=0.352, p=0.006) and pyrimidine base damage r=0.318, p=0.014), whereas no significant correlation was observed for purine base damage (p>0.05).

Table 4. Influences of GSTM1, GSTT1 and hOGG1 genotypes on oxidative DNA damage parameters in patients and controls. Values are mean±SD

	Genotype	CAD patients	Controls
Basal DNA damage	GSTM1 active	8.96±2.13	7.58±2.00
	GSTM1 null	9.80±3.16	8.59±3.30
	GSTT1 active	**9.32±2.74***	7.74±2.74
	GSTT1 null	9.96±3.08	9.62±1.94
	Ser/Ser	8.33±2.76	7.51±1.72
	Ser/Cys	10.55±2.46	7.56±2.01
	Cys/Cys	11.30±1.26	15.40±2.61
	Ser/Cys+Cys/Cys	**10.66±2.30****	8.76±3.55
Pyrimidine base damage (Endo III sites)	GSTM1 active	16.50±9.69	12.19±5.94
	GSTM1 null	18.32±7.52	17.39±11.47
	GSTT1 active	16.20±8.32	13.69±8.80
	GSTT1 null	22.83±6.78	19.25±10.63
	Ser/Ser	14.95±6.40	14.04±7.28
	Ser/Cys	18.48±8.83	15.77±12.48
	Cys/Cys	31.70±4.17	13.16±3.13
	Ser/Cys+Cys/Cys	20.37±9.51	15.37±11.47
Purine base damage (Fpg sites)	GSTM1 active	10.91±4.97	11.03±2.81
	GSTM1 null	16.50±5.62	17.00±7.81
	GSTT1 active	12.61±5.29	12.74±4.20
	GSTT1 null	20.23±4.56	19.19±12.06
	Ser/Ser	12.62±5.94	14.53±7.20
	Ser/Cys	15.09±5.75	13.03±5.68
	Cys/Cys	20.52±3.40	12.13±2.16
	Ser/Cys+Cys/Cys	15.86±5.73	12.89±5.23

*P=0.014 (vs controls); **P=0.022 (vs. controls); Mann-Whitney U test. CAD - coronary artery disease

Table 5. Correlation between oxidative DNA damage parameters and Gensini score

	Correlation coefficient (r)	Significance (P)
Basal DNA damage	0.352	**0.006**
Pyrimidine base damage (Endo III sites)	0.318	**0.014**
PBD, (Fpg sites)	0.110	0.408

Spearman's correlation test. PBD - purine base damage

Discussion

In the present study, we investigated two different types of biomarkers in CAD patients. We assessed a biomarker of effect (DNA damage) and a biomarker of susceptibility (gene polymorphism) for CAD risk. Our results significantly highlighted increased basal DNA damage levels in CAD patients with respect to controls. However, neither pyrimidine nor purine base damage levels, which reflect oxidative DNA damage, were significantly different between the groups. On the other hand, basal DNA and pyrimidine base damage levels were significantly correlated with disease severity based on Gensini scoring. Although there were some significant differences in terms of basal DNA damage between the patients and controls carrying the same alleles, genotype analysis did not reveal a clear association between any of the studied SNPs (GSTM1, GSTT1, and hOGG1) and CAD. Although the results of our study pointed out the effects of age, hypertension, and family history as possible risk factors for CAD, none of these factors affect the frequency of the polymorphisms after adjusting the results for age, gender, the presence of hypertension, and smoking habits.

The association between DNA damage and CAD was investigated in several studies, and there is increasing evidence that DNA damage plays an important role in atherogenesis. Botto et al. (5) investigated micronucleus (MN) frequency in CAD patients and found that MN levels were higher in these patients than in controls. They also observed a relationship between increased MN frequencies and disease severity. In a follow-up study, major adverse cardiovascular events (MACEs) such as heart failure, stroke, or cardiovascular death were reported in CAD patients for a period of 51 months, and MN levels were found to be significantly higher in patients who had MACEs than in those who did not (18). Many factors can cause DNA damage, including mainly oxidative stress. Oxidative stress to lipids and DNA caused by free radicals is known to be one of the several fundamental mechanisms that are responsible for developing atherosclerosis and cancer (19). Some possible atherogenic mechanisms, such as increasing ROS production, the potentiation of LDL oxidation, and the diminished release of nitric oxide, were suggested in various studies (20, 21). Oxidative stress-related studies in CAD patients have usually been performed by measuring the lipid peroxidation or antioxidant capacity in plasma (4, 22). However, the number of studies on oxidative DNA damage in CAD patients is limited, and the information in the literature regarding the role of DNA damage in the etiology of CAD is inadequate. In the present study, we observed significantly increased levels of basal DNA damage but not oxidative DNA damage in the lymphocytes of CAD patients. This finding indicates possible damage to the DNA of these patients; however, this damage, as detected by the modified comet assay, was not a result of oxidized DNA bases. Moreover, basal DNA damage levels in patients were positively correlated with disease severity. Similar studies using the alkaline comet assay have been previously conducted for investigating oxidative DNA damage in CAD patients. Botto et al. (6) demonstrated that increased levels of SSBs and oxidized DNA bases correlated with the severity of CAD in patients. Demirbağ et al. (4, 23) investigated DNA damage using the standard alkaline comet assay (not modi-

fied with repair enzymes) in CAD patients and reported higher SSBs and lower plasma total antioxidant capacity levels in patients with acute coronary syndrome than those in patients with stable angina and healthy subjects. In another study, contrary to our findings, significantly increased levels of oxidized pyrimidine and purine bases were found; however, no significant difference was reported in terms of SSBs between CAD patients and controls (24). Recently, Rajesh et al. (3) assessed DNA damage using the standard comet assay in a rather large population of CAD patients and reported significantly higher levels of DNA damage as well as malondialdehyde (MDA) and nitrite/nitrate levels in patients than those in controls.

In the present study, the roles of GSTM1, GSTT1, and hOGG1 gene polymorphisms and their relationship with oxidative DNA damage were also investigated in CAD patients. The GSTM1 null genotype is very common among Caucasians and is found in approximately 50% of the population (25). A homozygous deletion of the GSTT1 gene (null genotype) is found in 10%–20% of Caucasians, making them unable to perform GSTT1-mediated detoxification reactions (26). Genes coding for DNA repair molecules, such as hOGG1, have been proposed as candidate susceptibility genes because of the importance of maintaining genomic integrity. (11). Our results regarding the frequencies of studied gene polymorphisms are consistent with those of previous Turkish studies (27, 28). However, our results did not show any statistical difference between CAD patients and controls in terms of GSTM1, GSTT1, and hOGG1 variant allele frequency. There are some previous studies investigating the effect of these polymorphisms on the risk of developing CAD, and the findings are contradictory. In a recent study, similar to our findings, the frequency of the GSTM1 and GSTT1 null alleles did not differ among CAD patients and controls; however, a combined effect of CYP1A1 and GSTT1 variant genotypes was reported for the risk of developing CAD (7). Similarly, Bazo et al. (29) did not detect any association between GSTM1 and GSTT1 gene polymorphisms and coronary atherosclerosis in a Brazilian population. On the other hand, Girisha et al. (30) reported a decreased frequency of the GSTT1 null genotype and no significant association with GSTM1 genotypes in CAD patients compared with in controls. An association between GST polymorphisms and CAD risk is demonstrated mostly in studies investigating the interaction of these polymorphisms with cigarette smoking. In three different studies, a strengthened effect of GST-null genotypes on smoking-induced CAD risk was reported, although GST null genotypes did not differ between patients and controls (10, 31, 32). Similar results were obtained in a large cohort study pointing out a positive interaction with the GSTT1 null genotype (8).

hOGG1 is one of the polymorphic repair genes in the BER pathway, which has a critical role in removing damaged DNA bases. It catalyzes the cleavage of the glycosylic bond between the modified base and the sugar moiety; as a result, an abasic apurinic or apyrimidinic (AP) site occurs in the DNA. Studies on the role of hOGG1 gene polymorphism in CAD risk are limited. Two recent studies on a Taiwanese population demonstrated contradictory results. Wang et al. (33) reported an association between hOGG1 polymorphism and an increased risk of CAD, whereas Shyu et al. (34) found no association. In a cohort study, 98 subjects suffering from severe atherosclerotic lesions were followed up for 14 years, and an adverse effect on survival was detected in subjects having the double GSTM1 and GSTT1 deletion, although the frequencies of both null alleles were not different from the general population. In the same study, the survival rate was not influenced by hOGG1 polymorphism (35). Gökkuşu et al. (36) investigated the role of different DNA repair enzyme polymorphisms in progressing CAD, and they reported that XRCC3 and hOGG1 genetic variants may be risk factors that may lead to the development of CAD. Another study on a Chinese population revealed similar results in coronary ectasia patients, with a significant association of hOGG1 Ser326Cys polymorphism. The study also reported higher 8-OHdG levels in subjects carrying the hOGG1 variant allele (37).

During the past few years, a large number of studies have focused on individual susceptibility for predicting the risk of developing some common diseases such as cancer and atherosclerosis. A variety of factors can contribute to variations in the individual response to environmental mutagenic agents, thus causing some individuals to be susceptible to these disease and others to be resistant. Moreover, combinations of biomarkers are valuable in assessing the individual risk of diseases (38, 39).

Study limitations

The small sample size limited us from analyzing more factors involving the risk of CAD, such as smoking. Therefore, these preliminary findings need to be validated in larger populations.

Conclusion

In this study, we demonstrated the role of DNA damage in CAD; however, no significant differences were observed in terms of oxidized DNA bases between patients and controls. Therefore, we can conclude that the DNA damage we observed does not originate from ROS. In the present study, the studied genotypes (GSTM1, GSTT1, and hOGG1) showed similar frequencies between groups. Although there were some significant differences in terms of basal DNA damage between the patients and controls carrying the same alleles, genotype analysis did not reveal a clear association between any of the studied SNPs and CAD.

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From Prof. Dr. Arif Aksit's collection

Complex interplay of DNA damage, DNA repair genes, and oxidative stress in coronary artery disease

Oxidative stress and DNA damage have been increasingly recognized to coexist in the setting of coronary artery disease (CAD). DNA damage is present in all cells within the atherosclerotic plaque, and there is increasing evidence that human atherosclerosis is associated with damage to DNA of both circulating cells and cells of the vessel wall. DNA damage usually includes DNA strand breaks, mutations of single bases, modified bases (including oxidation), or DNA adducts. Many of the risk factors associated with atherogenesis, such as smoking and diabetes mellitus, could directly induce DNA damage (1).

Inflammation plays a pivotal role in atherogenesis. The response is continuously mediated by monocyte-derived macrophages and specific subtypes of T lymphocytes at every stage of the disease. Granulocytes are rarely implicated because they are scarce in atherosclerotic lesions during any phase of atherogenesis (2). The action of different subsets of macrophages in atherosclerotic lesions is subsequently regulated by cytokines released by T cells. The monoclonal origin of cells from human atherosclerotic plaques is the triggering and the substantiating event for the association of DNA damage with the development of cardiovascular pathologies in the general population (3).

In addition to traditional CAD risk factors, oxidative stress has been regarded as one of the most important contributors to the progression of atherosclerosis. Oxidative stress could also constitute the major causative mechanism for DNA damage in CAD (4). Reactive oxygen species include the superoxide anion, hydrogen peroxide, hydroxyl radical, peroxynitrite, and lipid peroxides. Superoxide and hydrogen peroxide are normally not reactive to DNA, but they can be converted via the Fenton reaction to the hydroxyl radical, which is extremely reactive. The hydroxyl radical can induce a vast array of damage to both nuclear and mitochondrial DNA (1, 5). Apart from oxidative stress, inflammation is another major determinant of DNA damage along with ischemia reperfusion.

Kadıoğlu et al. (6 in this issue of AJC entitled "The role of Oxidative DNA damage, GSTM1, GSTT1 and hOGG1 gene polymorphisms in coronary artery disease risk.") provided evidence of significantly increased DNA damage in peripheral blood lymphocytes of CAD patients compared with healthy subjects, which was in agreement with previous studies (7, 8). However, they showed that DNA damage was not the result of augmented oxidative stress in those patients leaving inflammation as the most probable cause.

In atherosclerotic plaques, there is evidence of activation of DNA repair mechanisms along with signs of DNA damage. Genome lesions are eliminated with DNA strand break repair, base excision repair, and mismatch repair, whereas patients with specific polymorphisms in genes responsible for DNA repair have been found to be more susceptible to CAD (9, 10). The genotype analysis by Kadıoğlu et al. (6) revealed no clear association between the studied hOGG1 gene polymorphism and CAD. Nevertheless, the dual heterogeneity of risk factors and of the CAD patients should be noted. Therefore, further work is needed in delineating the relationship between DNA repair gene polymorphisms and CAD.

Elena Vakonaki, Konstantinos Tsarouhas[1], Demetrios A. Spandidos*, Aristidis M. Tsatsakis
Center of Toxicology Science and Research, *Laboratory of Clinical Virology, Faculty of Medicine, University of Crete; Heraklion-*Greece*
[1]Cardiology Clinic, University General Hospital of Larisa; Lárisa, Thessaly-*Greece*

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Address for correspondence: Aristidis M. Tsatsakis, PhD, DSc, ERT, Head of Forensic Sciences and Toxicology Department
Medical School, University of Crete, 71003 Heraklion, Crete,
P.O. Box 1393-*Greece*
Phone: +30(2810)-394870 Fax: +30(2810)-542098 E-mail: tsatsaka@uoc.gr
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