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The impact of OGG1, MTH1 and MnSOD gene polymorphisms on 8-hydroxy-2′-deoxyguanosine and cellular superoxide dismutase activity in myocardial ischemia–reperfusion

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Abstract Ischemia–reperfusion (I/R) injury, by inducing oxidative DNA damage, is one of the leading causes of increased patient morbidity and mortality in coronary artery by-pass grafting (CABG) surgery. 8-Hydroxyguanine (8-OHG) is an important oxidative base lesion. The 8-oxoguanine glycosylase (hOGG1) and hMTH1, which have several polymorphisms, remove 8-OHdG from the nucleotide pool. We investigated whether there are any correlations the biomarkers of oxidative stress (superoxide dismutase; SOD and 8-OHdG in serum) with genotype for two DNA repair genes (OGG1 and MTH1) and an antioxidant enzyme gene (manganese superoxide dismutase; MnSOD). Therefore, we measured DNA damage (8-hydroxy-2-deoxyguanosine; 8-OHdG) and endogenous antioxidant activity (SOD) at five different time points (T1, before anesthesia; T2, after anesthesia; T3, after ischemia; T4, after reperfusion and T5, after surgery). and also, MnSOD and MutT homolog 1 (MTH1) genes polymorphisms were genotyped by polymerase chain reaction–restricted fragment length polymorphism (PCR–RFLP) in patients undergoing coronary artery by-pass grafting (CABG) surgery. No statistically significant differences were detected in the levels of 8-OHdG and SOD in serum in terms of OGG1 Ser326Cys, MTH1 Val83Met and MnSOD Ala16Val genetic polymorphisms. Our results suggest that OGG1, MTH1 and MnSOD gene polymorphisms are not genetic risk factors for I/R injury.

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Introduction

Although open coronary artery by-pass grafting (CABG) surgery has become a routine procedure worldwide, patient morbidity and mortality due to adverse postoperative complications are still unacceptably high [1]. ROS, which are thought to be responsible from these adverse complications, including superoxide radical, hydroxyl radical and hydrogen peroxide have been shown to increase during CABG operation. Furthermore, several studies have proposed the essential role of ROS in the pathogenesis of myocardial ischemia–reperfusion injury [2, 3]. Mitochondria are the main source of ROS. Superoxide is the initial oxidant generated from NAD(P)H or other oxidases such as cytochrome P-450 and is also generated by the mitochondrial electron transport chain. ROS production is accompanied by the induction of natural defense and repair mechanisms and effect upon tissue's vitality. Superoxide dismutase (SOD) is a first line of defense against oxidative stress under physiological and pathological conditions [4]. SOD catalyses the dismutation reaction of the toxic superoxide radical to molecular oxygen and hydrogen peroxide and thus forms a crucial part of the cellular antioxidant defence mechanism. In most mammalian species, SOD appears to be the most active antioxidant enzyme in the myocardium. Manganese superoxide dismutase (MnSOD), a key mitochondrial antioxidant enzyme, is one important repair enzyme for reactive oxidative stress (ROS)-induced damage. MnSOD polymorphisms in the 9 position of the signal sequence of the

protein may lead to critical enzyme deficiency. In MnSOD polymorphism two genetic variants of MnSOD were identified. A structural mutation, a T to C substitution in the mitochondrial targeting sequence, was found that changes the amino acid codon at −9 position in the signal peptide from valine (GTT) to alanine (GCT) [5].

7,8-Dihydro-8-oxoguanine (8-OHdG) is one of the main DNA modifications produced by ROS. Because 8-OHdG can pair with cytosine and adenine bases during DNA synthesis, when 8-hydroxyguanine (8-OHG) is present in the DNA template, it causes G:C to T:A transversions [5]. On the other hand, 8-hydroxydeoxyguanine (8-OHdG) is one of the main DNA modifications produced by hydrogen peroxide. It is highly mutagenic in vitro and in vivo, yielding G:C to T:A transversion, since it directs the incorporation of cytosine and adenine nucleotides opposite the lesion during DNA replication and it induces A:T to C:G transversions when 8-hydroxy-dGTP in the nucleotide pool is incorporated into DNA [6, 7]. The hOGG1 gene encodes the DNA glycosylase that removes the mutagenic lesion 7,8-dihyro-8-oxoguanine (8-OHdG) from DNA. A frequently found polymorphism resulting in a serine to cysteine substitution at position 326 of the OGG1 protein has been associated in several molecular epidemiologic studies with cancer development [8, 9]. hMTH1 also removes 8-OHdG from the nucleotide pool [10]. Less is known about the consequences of CABG on the endogenous antioxidant capacity that is derived from the activity of antioxidant enzymes such as glutathione peroxidase (GPx) and SOD, responsible for the clearance of peroxides and superoxide, respectively.

In the present study, it was aimed (i) to measure the DNA damage (8-OHdG) and endogenous antioxidant activity (SOD) at the following stages of the intervention: before anesthesia (T1), after anesthesia (T2), after ischemia (T3), after reperfusion (T4) and after the surgery (T5) and reveal the differences among the time intervals (*no individual susceptibility*), (ii) to reveal individual susceptibility by investigating the impact of OGG1, MTH1 and MnSOD gene polymorphisms on serum 8-OHdG and SOD activity in patients undergoing coronary artery bypass grafting.

Materials and methods

Patient population

Sixty patients (18 women and 42 men) aged 30–75 years, with American Society of Anesthesiologists (ASA) physical status II and IV, who underwent elective CABG surgery were prospectively studied after obtaining approval from the Ataturk Training and Research Hospital ethics committee. The study was performed in accordance with the declaration of Helsinki. Their demographic data are reported in Table 2. Each patient was informed about the anaesthetic procedure and study protocol in detail at the preoperative visit and gave their written consent before inclusion in the study. Patients answered standardised health questionnaires about their medical history, occupational exposure, and lifestyle such as smoking and drug consumption to rule out confounding effects. Before surgery, patients underwent thorough cardiac diagnostics, including determination of ejection fraction. We excluded pregnant women and patients with hormone treatment, left ventricular ejection fraction of less than 40%, a history of previous cardiac operation, neuromuscular disorders, neurological, haematological, hepatic, renal, or severe pulmonary disease, a history of drug and alcohol abuse, patients undergoing combined operations (simultaneous valve repair, carotid endarterectomy, or left ventricular aneurysm repair) from the study.

Before surgery, patients underwent thorough cardiac diagnostics, including determination of ejection fraction. Age, body mass index (BMI), medical history, current medications, preoperative left ventricular ejection fraction (LVEF/%), and infarction time, if present, were recorded for each patient. All preoperative cardiac medication was continued until the morning of the surgery, except for angiotensin-converting enzyme (ACE) inhibitors.

Anaesthetic and surgical procedure

All patients were pre-medicated with oral diazepam (5 mg) the night before and intramuscular morphine sulphate (0.1 mg kg^{-1}) 45 min before the surgery. Noninvasive monitoring was established and radial artery was cannulated under local anaesthesia. This procedure enables internal monitoring of arterial tension and patient's metabolic and blood-gas status. It was also used to obtain blood samples required for polymorphism studies. Anaesthesia was similar for all patients, and consisted of 10 μg kg^{-1} fentanyl in combination with 0.3 mg kg^{-1} etomidate and 0.15 mg kg^{-1} cisatracurium besylate, given intravenously at induction. Intubation was performed after 2 min and anaesthesia was maintained by additional doses of fentanyl (5 μg kg^{-1}) and cisatracurium besylate (0.03 mg kg^{-1}), and by inhalation of 0.5–2% sevoflurane. The depth of anaesthesia during surgery was between 40 and 50, according to the bispectal index (BIS XP™; Aspect Medical Systems, Newton, MA). Ventilation during the anaesthesia was performed in a volume-controlled mode. In surgery, a median sternotomy was made, heparin was given (350 IU kg^{-1}), aortic and right atrial venous cannula were inserted, and a standard cardiopulmonary bypass (CPB) with moderate systemic hypothermia (28 to 30°C of nasopharynx temperature) was instituted. Activated

coagulation time (ACT) was kept above 450 s throughout the CPB period. Standard pump priming solution was used in each patient. Routine surgical technique and cardioprotective strategies were used in all patients. For myocardial protection, the aorta was cross-clamped and cold anterograde and retrograde intermittent cardioplegia (Plegisol® Abbott, North Chicago, IL, ABD) were used. Vasopressors and vasodilators were administered as necessary. Hematocrit concentrations were maintained between 20 and 25%. After the surgical procedure, by reperfusion and rewarming of the heart to temperature of 36°C, the heart was placed in atrioventricular mode at a rate of 90 bpm, and the patients were separated from CPB. After removal of the aortic cannula, heparin activity was neutralised with protamine at a ratio of 1 mg of protamine per 100 U heparin. Protamine administration was further guided by ACT measurements aiming at 140 s. When mean arterial pressure was below 60 mmHg, vasopressor therapy was started. During surgery, we recorded the duration of aortic clamping and CPB, volume of cardioplegic solution used, volume of haemodilution and infusions, diuresis, and minimum body temperature. At the end of the surgical procedure, patients were transferred to the intensive care unit. When haemodynamically stable and rewarmed, the patients were weaned from the ventilator and extubated.

Blood sampling

The serum samples were collected at the following stages of the intervention: the reference level (T1) was obtained immediately after cannulation of the arterial system and before anaesthesia; the second sample (T2) was taken 20 min after anaesthesia was introduced; the third sample (T3) was taken after 20 min of the aortic cross-clamp; the fourth sample (T4) was taken 20 min after the removal of the aortic cross-clamp; and the final sample (T5) was obtained by venipuncture 24 h after the surgery. At the same time points, hemoglobin, sodium, potassium, glucose, lactate and bicarbonate were determined in blood samples taken from the radial artery. Venous blood samples, taken in EDTA for genotyping analysis, were obtained during routine blood sampling for biochemical and hematological analyses from the patients. OGG1 Ser326Cys, MTH1 Val83Met and MnSOD Ala9Val polymorphisms were genotyped by restriction fragment length polymorphism–polymerase chain reaction (RFLP–PCR).

DNA isolation

Genomic DNA was isolated from blood using a commercial DNA extraction kit according to manufacturer's recommendations (Blood DNA Extraction Kit, Cat No: GF-BD-050, Vivantis).

OGG1, MTH1 and MnSOD Genotyping

A 207 bp fragment was amplified by PCR in a 30 µl reaction volume that contained 100 ng genomic DNA, 0.2 mM of dNTP, 1.5 mM of $MgCl_2$, 0.3 pmol of each primer and 1 U of *Taq DNA polymerase* for OGG1 genotyping. A 247 bp fragment was amplified by PCR in a 25 µl reaction volume that contained 100 ng genomic DNA, 0.2 mM of dNTP, 1.5 mM of $MgCl_2$, 0.2 µM of each primer and 1.5 U of *Taq DNA polymerase* for MTH1 genotyping. For MnSOD genotyping, a 172 bp fragment was amplified by PCR in a 25 µl reaction volume that contained 100 ng genomic DNA, 0.25 mM of dNTP, 1.5 mM of $MgCl_2$, 0.3 µM of each primer and 1 U of *Taq DNA polymerase*. Primers, cycling conditions, restriction enzymes and incubation conditions for gene polymorphisms are presented in Table 1. All digested products were electrophoresed on a 3% agarose gels and visualized under UV light with ethidium bromide.

Measurement of SOD activity

SOD activity in serum was measured by a colorimetric method with a commercial kit (SOD Activity Assay Kit, Cat No: K335-100, Biovision) according to the manufacturer's instructions.

Measurement of the levels of serum 8-hydroxy-2′-deoxyguanosine (8-OHdG)

The levels of serum 8-OHdG were measured by Enzyme Linked Immunosorbent Assay (ELISA) (StressXpress DNA Damage ELISA Kit, EKS-350, Stressgen) according to the manufacturer's instructions.

Statistical analysis

Data analysis was performed by using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, United States). Whether the distributions of continuous variables were normally or not was determined by using Shapiro–Wilk test. Data were shown as median (IQR). The differences between groups were compared by using Mann–Whitney *U* test. A *P* value less than 0.05 was considered statistically significant. Moreover, whether the differences among time intervals were statistically significant or not was evaluated by Friedman test. When the *P* value from the Friedman test is statistically significant to know which time differs from which others, Bonferroni Adjusted Wilcoxon Sign Rank test was used.

Table 1 RFLP–PCR genotyping analysis for polymorphisms on OGG1, MTH1 and MnSOD genes

Genetic polymorphism	Primer sequence (5′F–3′R)	Cycling conditions						Restriction enzyme/incubation temperature/incubation time	Fragment size (bp)	Reference
		In. den.	Den.	Ann.	Elon.	Ext.	Cy. no.			
OGG1 Ser326Cys	ACT GTC ACT AGT CTC ACC AG TGA ATT CGG AAG GTG CTT GGG GAA T	94°C for 2 min	94°C for 15 s	60°C for 30 s	72°C for 35 s	72°C for 10 min	30 cycles	*Fnu*4HI/37°C/16 h	207	[11]
MTH1 Val83Met	CAT GGC ACC ATG CCC TGA GAG ATG GGA CCC GCA TAG	94°C for 5 min	94°C for 1 min	60°C for 1 min	72°C for 1 min	72°C for 5 min	30 cycles	*Nsi*I/37°C/16 h	247	[12]
MnSOD Ala16Val	CAG CCC AGC CTG CGT AGA CGG GCG TTG ATG TGA GGT TCC AG	94°C for 2 min	94°C for 1 min	60°C for 1 min	72°C for 1 min	72°C for 7 min	33 cycles	*Bsa*WI/60°C/16 h	172	[13]

In. den. initial denaturation, *Den.* denaturation, *Ann.* annealing, *Elon.* elongation, *Ext.* extension, *Cy. no.* cycle number

Results

The frequencies of OGG1, MTH1 and MnSOD allele and genotype were estimated with CABG surgery in this study. Table 2 displays the baseline characteristics and operative data of 60 CABG patients. The participants were predominantly male (n = 42, 70%) and nonsmokers (n = 39, 65%). OGG1 Ser326Cys, MTH1 Val83Met and MnSOD Ala16Val genotype distributions and their allele frequencies in the entire study population are summarized in Table 3. We found that the frequencies of OGG1 Ser/Ser, Ser/Cys and Cys/Cys genotypes were 55, 42 and 3%, respectively; while the frequencies of MnSOD Ala/Ala, Ala/Val and Val/Val genotypes were 52.6, 42.1 and 5.3%, respectively. The frequencies of MTH1 Val83Val and Val83Met genotypes also are 93.3 and 6.7%.

Figure 1 summarizes the levels of 8-OHdG, as independently from genotypes, at 5 predetermined time intervals (as T1: before anesthesia induction, T2: after anesthesia induction, T3: at aortic cross-clamping, T4: during reperfusion and T5: at 24 h of operation). The levels of 8-OHdG at T3, T4 time periods were decreased significantly compared to T1, while the difference between T2 and T3, T4 time periods as well as between T3–T5 and T4–T5 were significant, pointing out that the lowest 8-OHdG level obtained at T3 might be the result of repair induced by ischemia for compensation of oxidative stress ($P < 0.001$). However, an upward incline at T4–T5 time periods is also remarkable. The enzyme activity of the antioxidant SOD in full blood at

Table 2 Baseline characteristics and operative data of 60 CABG patients

Parameters	Mean ± SD
Age (years)	61.10 ± 8.71
Gender n (%)	
Female	18 (30)
Male	42 (70)
BMI	28.75 ± 4.67
Cigarette status n (%)	
Nonsmokers	39 (65)
Smokers	21 (35)
Smokers	
Cigarettes/day	18.95 ± 10.91
Smoking duration	
Year	33.45 ± 13.71
Preoperative ejection fraction (%)	50.02 ± 11.23
Pump duration	
Min	77.12 ± 22.00
Duration of cross-clamp (ischemia)	
Min	48.22 ± 14.19
Vasopressor need n (%)	7 (11.6)

Table 3 OGG1 Ser326Cys, MTH1Val83Met and MnSOD Ala16Val Genotype Distributions and their Allele Frequencies

				Alelle frequency (%)	
OGG1Ser326Cys genotype, n (%)					
Ser326Ser	Ser326Cys	Cys326Cys	Ser326Cys + Cys326Cys	Ser326	Cys326
33 (55)	25 (42)	2 (3)	27 (45)	76	24
MTH1 genotype Val83Met, n (%)					
Val83Val	Val83Met	Met83Met	Val83Met + Met83Met	Val83	Met83
56 (93.3)	4 (6.7)	0 (0)	2 (3.3)	97	3
MnSOD Ala16Val genotype, n (%)					
Ala16Ala	Ala16Val	Val16Val	Ala16Val + Val16Val	Ala16	Val16
30 (52.6)	24 (42.1)	3 (5.3)	27 (47.4)	74	26

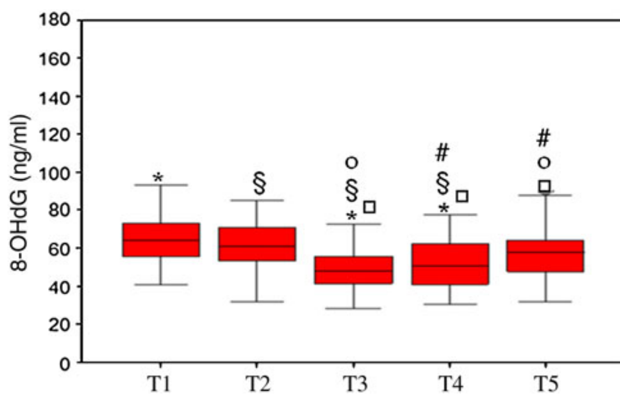

Fig. 1 Serum 8-OHdG (ng/ml) levels in five different time intervals (T1–T5) not taking account into genotypes (T1–T5). *$P < 0.001$; T1–T3, T1–T4; §$P < 0.001$; T2–T3, T2–T4; □$P < 0.001$, T3–T5, T4–T5 Anova

Table 4 The levels of serum 8-OHdG (ng/ml) and the activity of SOD in five different time intervals not taking account into genotypes (T1–T5)

Five different time intervals	8OHdG (ng/ml) Median (IQR)	SOD activity Median (IQR)
Before operation (T1)	64.16	23.30
After anesthesia (T2)	61.19	22.87
After ischemia (T3)	48.18	23.17
After reperfusion (T4)	50.76	41.35
After operation (T5)	57.92	32.53

20 min after reperfusion (T4) reached a maximum rise compared to the levels obtained at T1, T2, and T3 ($P < 0.001$), however, afterwards this increase showed a decline until the end of 24 h ($P < 0.001$) (Fig. 2).

When it was checked whether genotypes had impacts on the levels of 8-OHdG, for OGG1, 8OHdG activities which are measured in homozygotes at T1, T2, T3, T4 and T5 were not significantly different from those in heterozygous/mutant ones ($P > 0.05$) (Table 4). Based on the genotype, we obtained the lowest 8-OHdG level at T3 as a result of ischemia induced by aortic cross-clamping.

Table 5 summarizes the effect of genotype on the levels of 8-OHdG for MTH1 Val83Met. The levels at predetermined time intervals are not different between the homozygote and heterozygous/mutant individuals ($P > 0.05$). The levels of 8OHdG in patients carrying heterozygous/mutant variant at T3 were higher than in patients carrying homozygotes, but this increase was not statistically significant. It suggested that the lack of repair by OGG1 for 8-OHdG caused the increase of the levels of 8-OHdG. This finding showed the importance of genetic background of patients. For gene–gene (OGG1-MTH1), in contrary of our

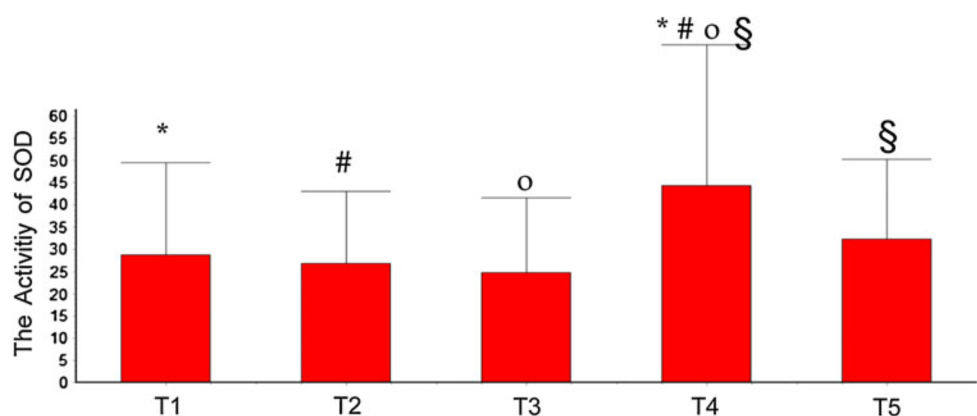

Fig. 2 The activities of SOD in five different time intervals (T1–T5) not taking account into genotypes (T1–T5). *$P < 0.001$; T1–T4; #$P < 0.001$; T2–T4; °$P < 0.001$, T3-T4; □$P < 0.001$, T4–T5 Anova

Table 5 The levels of serum 8-OHdG (ng/ml) in five different time intervals (T1–T5) in terms of OGG1 Ser326Cys genotype

Five different time intervals	8OHdG (ng/ml) Median (IQR)
Before operation (T1)	
Wild	63.8 (16.49)
Heterozygote + mutant	64.4 (25.94)
P value	0.812
After anesthesia (T2)	
Wild	62.4 (15.42)
Heterozygote + mutant	61.2 (20.99)
P value	0.941
After ischemia (T3)	
Wild	48.9 (16.84)
Heterozygote + mutant	46.3 (13.18)
P value	0.629
After reperfusion (T4)	
Wild	51.4 (19.11)
Heterozygote + mutant	49.9 (26.96)
P value	0.345
After operation (T5)	
Wild	61.0 (16.88)
Heterozygote + mutant	53.2 (15.49)
P value	0.143

expectations, no inter-group (T1-T5) differences were found with respect to the levels of 8OHdG, for both OGG1 Ser326Cys and MTH1 Val83Met genotypes which play role in repair of the DNA damage (8-OHdG) ($P > 0.05$) (Table 6). Table 7 shows the activity of SOD in five different time intervals (T1–T5) in terms of MnSOD Ala16Val genetic polymorphism. The activities of SOD in patients carrying heterozygous/mutant variants were decreased in predetermined time intervals compared to patients carrying wild, except T2, however these decreases were not statistically significant. A highest decrease was observed in T4, reperfusion consumed SOD activity even if that was not statistically significant, this trend might give an idea about the consuming of SOD, especially at T4 and provide comparable data at other time points (Table 8).

Discussion

This study aimed at gaining further insight into the short-term effects of oxidative stress on the changes in endogenous antioxidant capacity and 8-OHG, which is a sensitive biomarker of DNA damage, in patients undergoing CABG by sevoflurane anesthesia and moreover, the impact of individual susceptibility on ischemia–reperfusion injury in

Table 6 The levels of serum 8-OHdG (ng/ml) in five different time intervals (T1–T5) in terms of MTH1 Val83Met genotype

Five different time intervals	8OHdG (ng/ml) Median (IQR)
Before operation (T1)	
Wild	64.7 (16.97)
Heterozygote + mutant	52.2 (33.77)
P value	0.175
After anesthesia (T2)	
Wild	61.2 (16.77)
Heterozygote + mutant	61.1 (32.30)
P value	0.966
After ischemia (T3)	
Wild	48.2 (14.15)
Heterozygote + mutant	51.6 (34.86)
P value	0.764
After reperfusion (T4)	
Wild	51.4 (22.22)
Heterozygote + mutant	48.0 (21.15)
P value	0.721
After operation (T5)	
Wild	59.5 (16.89)
Heterozygote + mutant	51.6 (10.72)
P value	0.228

Table 7 The levels of serum 8-OHdG (ng/ml) and the activity of SOD in five different time intervals (T1–T5) in terms of OGG1 Ser326Cys plus MTH1 Val83Met genotypes

Five different time intervals	8OHdG (ng/ml) Median (IQR)
Before operation (T1)	
Wild (OGG1 + MTH1)	64.8 (16.05)
Heterozygote + mutant (OGG1 + MTH1)	64.1 (25.52)
P value	0.510
After anesthesia (T2)	
Wild (OGG1 + MTH1)	63.2 (14.84)
Heterozygote + mutant (OGG1 + MTH1)	61.0 (20.80)
P value	0.662
After ischemia (T3)	
Wild (OGG1 + MTH1)	49.0 (16.91)
Heterozygote + mutant (OGG1 + MTH1)	46.5 (12,99)
P value	0.604
After reperfusion (T4)	
Wild (OGG1 + MTH1)	51,4 (19.75)
Heterozygote + mutant (OGG1 + MTH1)	49.1 (25.34)
P value	0.286
After operation (T5)	
Wild (OGG1 + MTH1)	61.1 (15.59)
Heterozygote + mutant (OGG1 + MTH1)	52.8 (15.59)
P value	0.078

Table 8 The activity of SOD in five different time intervals (T1–T5) in terms of MnSOD Ala16Val genetic polymorphism

Five different time intervals	SOD activity Median (IQR)
Before operation (T1)	
Wild	28.4 (38.51)
Heterozygote + mutant	21.6 (24.02)
P value	0.361
After anesthesia (T2)	
Wild	22.9 (19,34)
Heterozygote + mutant	23.3 (32.38)
P value	0.846
After ischemia (T3)	
Wild	23.1 (21.35)
Heterozygote + mutant	22.2 (17.20)
P value	0.719
After reperfusion (T4)	
Wild	46.5 (28.62)
Heterozygote + mutant	41.1 (18.88)
P value	0.846
After operation (T5)	
Wild	33.1 (26.07)
Heterozygote + mutant	32.5 (28.28)
P value	0.522

terms of their genetic polymorphisms was revealed by comparisons among time intervals.

The systemic increase in oxidative stress during CABG is well-documented but the various components of the oxidant–antioxidant balance and the contribution of the various mechanisms involved have not been fully evaluated yet [14]. Extracorporeal circulation, by increasing contact of blood with foreign substances, will induce systemic inflammatory responses associated with complement activation, cytokine release and cellular activation of neutrophils [15]. Protective and repair mechanisms are normally present in the cell to prevent DNA damage and the negative impact on endogenous antioxidant capacity from ROS during CABG process and these defense and repair mechanisms include the antioxidant enzymes (such as SOD, catalase and GPx) and DNA repair systems (such as BER, NER, etc.). Single Nucleotide Polymorphisms (SNPs) on genes, which play role in these mechanisms, can give rise to individual gene variants that can alter susceptibility to many pathological and physiological states. Given that most individuals show extensive sequence variation in their DNA repair and antioxidant genes, it is likely that susceptibility will vary between individuals depending on the particular combination of alleles inherited. We found that the distribution of the OGG1 Ser326-Cys, MTH1 Val83Met and MnSOD Ala16Val genotypes in the study population were as 42, 6.7 and 42.1%, respectively. The frequencies of these genotypes are similar to the results obtained from studies of Caucasians [11–13].

Nagayoshi et al. [16] found that urinary levels of 8-OHG were higher in cardiac patients than in non-cardiac patients after assessment of serial changes in oxidative stress of patients with acute myocardial infarction. However, they only evaluated systemic changes in 8-OHdG, not the changes at different time intervals. In our study, we found a slight but not statistically significant decrease in the levels of serum 8-OHG and the activity of SOD after ischaemia that may be associated with oxygen radicals formed. The enzyme activity of SOD showed a maximum rise at 20 min after reperfusion. It suggested that large quantities of ROS released during reperfusion damage proteins responsible for intracellular homeostasis producing tissue injury. Verma et al. [17] demonstrated that this injury can be further exacerbated during open heart surgery when the myocardium is exposed to global ischemic cardioplegic arrest. Furthermore, several studies have pointed to strategies attenuating reperfusion injury and systemic inflammatory response during open heart surgery to improve clinical outcome [18, 19]. Acute preoperative hemodilution may be associated with reduced effects of aortic cross-clamping and improved myocardial recovery in these patients. However, study in higher-risk patients with poor ventricular function and those requiring complex cardiac surgery, which are not the interest of our study, could contribute to the results.

OGG1 is the key enzyme for recognition and initial excision of the most common form of oxidative DNA base damage, 8-OHG. OGG1 gene is highly polymorphic with the most studied SNP being at codon 326 (Ser326Cys). It is not clear whether the contribution of this polymorphism affects the catalytic properties of the enzyme, and thus limited knowledge is available on the association between a variety of susceptibilities to different disease states and SNPs in this critical DNA repair gene. Homozygous carriers of the variant form of the OGG1 Ser326Cys gene appear to have reduced repair capacity for oxidized DNA lesions [20].

In the current literature, most epidemiological studies of the OGG1 Ser326Cys polymorphism are in relation to cancer. In the aspect of lung cancer, some authors could not demonstrate any association due to the polymorphisms of OGG1 Ser326Cys [21–23]. However, Karahalil et al. found a significant decrease in the risk of lung cancer for the Ser326Cys genotype, disappearing after adjustments for age, sex, and smoking as reported similarly by De Ruyck et al. [24]. The authors explained this as a result of proteins involved and up-regulated by oxidative stress for the repair and prevention of 8-OHG-derived mutations. Paz-Elizur et al. [25] also demonstrated that low OGG is associated

with high risk of head and neck cancer. The studies on association with ischemia–reperfusion injury induced 8-OHG and the DNA-repair capacity of an individual are limited. In a study by You et al. [26], in the ischemic-reperfused rat hearts exposed to oxidative stress induced by high levels of oxygen free radicals, both the formation of 8-OHG in DNA and the level of its repair process, 8-OHG endonuclease activity was found to be increased. In another experimental model, the accumulation of 8-OHG in nuclear DNA and altered level of OGG1 expression, as a pathogenic response to necrosis of renal tubular cells during ischemia–reperfusion injury of the kidney demonstrated the association between OGG1 and 8-OHG [27].

We also studied MTH1 Val83Met polymorphism. Consequently, it may be difficult to interpret the findings of our study in the aspect of MTH polymorphism. One reason for this might be the limited number of studies because of the infrequent number of variants of MTH. However, in our study, we tried to investigate the combined effect of the two polymorphisms on the detection of oxidative DNA damage biomarker and cellular SOD activity. Nevertheless, we examined the two DNA repair enzymes responsible from the same activity. The hypothesis of our study was to determine whether this combined action could act synergistically in oxidative DNA damage etiology. We have to acknowledge that the study did not yield statistically significant results, but none of the reports up to date have attempted to show the relationship between OGG1, MTH1 and MNSOD gene polymorphisms on 8-OHG and SOD activity in myocardial ischemia reperfusion research on clinical basis. Thus, to our knowledge, this is the first study to evaluate the prevalence of three polymorphisms in a Caucasian population or to examine the associations between the polymorphisms and ischemia reperfusion injury.

SOD, is the key enzyme involved in the detoxification of superoxide radicals. There are a few studies investigating the association of cancer and chronic illnesses with genetic polymorphisms in MnSOD gene [28]. Isbir et al. investigated the effect of MnSOD genetic polymorphism on cytokine release and MnSOD dismutase in patients undergoing CABG with CPB. Their results showed that baseline IL-6 did not differ between patients with different MnSOD genotypes but IL-6 levels were significantly higher in all patients but more significantly in V(VV + AV) carriers ($P = 0.003$). The MnSOD ValVal genotype was associated with significantly lower preoperative MnSOD levels compared to the AA carriers ($P < 0.05$). They concluded that MnSOD Ala16Val polymorphism influences IL-6 production and baseline MnSOD activity [29]. These epidemiological studies have yielded mixed results with either a weak or strong association in between. Since there is no study addressing the association between ischemia–reperfusion injury and polymorphisms of OGG1, MTH1 and MnSOD in open heart surgery patients, we conducted this study to investigate the role of individual susceptibility in these patients. We did not find significant differences in the levels of serum 8-OHG and the activity of SOD between sampling times. We assumed that active repair of endogenous oxidative DNA lesions took place in the myocardium, which survived the insults of ischemia and reperfusion. We further believe that repair of oxidative base lesions in the myocardium was associated with induced repair activity, which is attributed to the over expression and activation of some repair enzymes, especially OGG1. Results from these analyses may be affected by sources of bias and low participation rates that are common to clinical based studies. Of concern are the limited sample numbers in this study.

Conclusions

In conclusion, our experiments performed in a small number of selected patients, could not show a significant association between the OGG1 Ser326Cys, MTH1 Val83-Met, MnSOD Ala16Val polymorphisms and oxidative response to ischemia–reperfusion injury. These polymorphisms cannot explain individual variation in susceptibility in ischemia–reperfusion related tissue injury in humans. However, it is well documented that a close relationship existing between the DNA damaging activity of endogenous reactive ROSs and their effects on the microcirculation results in rapid increases of both serum 8-OHG content and SOD activity during CABG operations. Based on the data obtained in this study and in the light of relevant observations in the literature, larger case–control and prospective epidemiologic studies are needed to prove that reduced OGG1, MTH1 and MnSOD are indeed risk factors for patients in situations in which ischemia and reperfusion occur, such as cardioplegia during cardiac surgery.

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References

1. Venardos KM, Perkins A, Headrick J, Kaye DM (2007) Myocardial ischemia-reperfusion injury, antioxidant enzyme systems, and selenium: a review. Curr Med Chem 14:1539–1549
2. Kevin LG, Novalija E, Stowe DF (2005) Reactive oxygen species as mediators of cardiac injury and protection: the relevance to anesthesia practice. Anesth Analg 101:1275–1287
3. Dirksen MT, Laarman GJ, Simoons ML, Duncker DJ (2007) Reperfusion injury in humans: a review of clinical trials on reperfusion injury inhibitory strategies. Cardiovasc Res 74:343–355

4. Batinic-Haberle I, Reboucas JS, Spasojevich I (2010) Superoxide dismutase mimics: chemistry, pharmacology and therapeutic potential. Antioxid Redox Signal 13:877–918
5. Ambrosone CB, Freudenheim JL, Thompson PA, Bowman E, Vena JE, Marshall JR, Graham S, Laughlin R, Nemoto T, Shields PG (1999) Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, risk of breast cancer. Cancer Res 59:602–606
6. Shibutani S, Takeshita M, Grollman AP (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. Nature 349:431–434
7. Ishibashi T, Hayakawa H, Sekiguchi M (2003) A novel mechanism for preventing mutations caused by oxidation of guanine nucleotides. EMBO Rep 4:479–483
8. Bravard A, Vacher M, Moritz E, Vaslin L, Hall J, Epe B, Radicella JP (2009) Oxidation status of human OGG1-S326C polymorphic variant determines cellular DNA repair capacity. Cancer Res 69:3642–3649
9. Karahalil B, Kocabas NA, Ozçelik T (2006) DNA repair gene polymorphisms and bladder cancer susceptibility in a Turkish population. Anticancer Res 26:4955–4958
10. Speina E, Arczewska KD, Gackowski D, Zielińska M, Siomek A, Kowalewski J, Oliński R, Tudek B, Kuśmierek JT (2005) Contribution of hMTH1 to the maintenance of 8-oxoguanine levels in lung DNA of non-small-cell lung cancer patients. J Natl Cancer Inst 97:384–395
11. Karahalil B, Kocabaş NA (2005) hOGG1 SER326CYS genetic polymorphism in a Turkish population. Arch Toxicol 79:377–380
12. Miyako K, Kohno H, Ihara K, Kuromaru R, Matsuura N, Hara T (2004) Association study of human MTH1 gene polymorphisms with type 1 diabetes mellitus. Endocrine J 51:493–498
13. Kadioglu E, Sardas S, Ergun M, Unal S, Karakaya AE (2010) The role of oxidative DNA damage, DNA repair, GSTM1, SOD2 and OGG1 polymorphisms in individual susceptibility to Barrett's esophagus. Toxicol Ind Health 26:67–79
14. Luyten CR, van Overveld FJ, De Backer LA, Sadowska AM, Rodrigus IE, De Hert SG, De Backer WA (2005) Antioxidant defence during cardiopulmonary bypass surgery. Eur J Cardiothorac Surg 27:611–616
15. Ohata T, Mitsuno M, Yamamura M, Tanaka H, Kobayashi Y, Ryomoto M, Yoshioka Y, Miyamoto Y (2007) Minimal cardiopulmonary bypass attenuates neutrophil activation and cytokine release in coronary artery bypass grafting. J Artif Organs 10:92–95
16. Nagayoshi Y, Kawano H, Hokamaki J, Miyamoto S, Kojima S, Shimomura H, Tsujita K, Sakamoto T, Yoshimura M, Ogawa H (2005) Urinary 8-hydroxy-2′-deoxyguanosine levels increase after reperfusion in acute myocardial infarction and may predict subsequent cardiac events. Am J Cardiol 95:514–517
17. Verma M, Maruvada P, Srivastava S (2004) Epigenetics and cancer. Crit Rev Clin Lab Sci 41:585–607
18. Suleiman MS, Zacharowski K, Angelini GD (2008) Inflammatory response and cardioprotection during open-heart surgery: the importance of anaesthetics. Br J Pharmacol 153:21–33
19. Conzen PF, Fischer S, Detter C, Peter K (2003) Sevoflurane provides greater protection of the myocardium than propofol in patients undergoing off-pump coronary artery bypass surgery. Anesthesiology 99:826–833
20. Karahalil B, Emerce E, Koçer B, Han S, Alkiş N, Karakaya AE (2008) The association of OGG1 Ser326Cys polymorphism and urinary 8-OHdG levels with lung cancer susceptibility: a hospital-based case-control study in Turkey. Arh Hig Rada Toksikol 59:241–250
21. Xu J, Zheng SL, Turner A, Isaacs SD, Wiley KE, Hawkins GA, Chang BL, Bleecker ER, Walsh PC, Meyers DA, Isaacs WB (2002) Associations between hOGG1 sequence variants and prostate cancer susceptibility. Cancer Res 62:2253–2257
22. Vogel U, Olsen A, Wallin H, Overvad K, Tjønneland A, Nexø BA (2004) No association between OGG1 Ser326Cys and risk of basal cell carcinoma. Cancer Epidemiol Biomarkers Prev 13:1680–1681
23. Kohno T, Kunitoh H, Toyama K, Yamamoto S, Kuchiba A, Saito D, Yanagitani N, Ishihara S, Saito R, Yokota J (2006) Association of the OGG1-Ser326Cys polymorphism with lung adenocarcinoma risk. Cancer Sci 97:724–728
24. De Ruyck K, Szaumkessel M, De Rudder I, Dehoorne A, Vral A, Claes K, Velghe A, Van Meerbeeck J, Thierens H (2007) Polymorphisms in base-excision repair and nucleotide-excision repair genes in relation to lung cancer risk. Mutat Res 631:101–110
25. Paz-Elizur T, Krupsky M, Blumenstein S, Elinger D, Schechtman E, Livneh Z (2003) DNA repair activity for oxidative damage and risk of lung cancer. J Natl Cancer Inst 95:1312–1319
26. You H, Kim G, Kim Y, Chun Y, Park J, Chung MH, Kim M (2000) Increased 8-hydroxyguanine formation and endonuclease activity for its repair in ischemic-reperfused hearts of rats. J Mol Cell Cardiol 32:1053–1059
27. Tsuruya K, Furuichi M, Tominaga Y, Shinozaki M, Tokumoto M, Yoshimitsu T, Fukuda K, Kanai H, Hirakata H, Iida M, Nakabeppu Y (2003) Accumulation of 8-oxoguanine in the cellular DNA and the alteration of the OGG1 expression during ischemia-reperfusion injury in the rat kidney. DNA Repair (Amst) 2:211–229
28. Flekac M, Skrha J, Hilgertova J, Lacinova Z, Jarolimkova M (2008) Gene polymorphisms of superoxide dismutases and catalase in diabetes mellitus. BMC Med Genet 9:30–39
29. Isbir S, Ergen A, Yilmaz H, Tekeli A, Arsan S (2008) Effect of Ala16Val genetic polymorphism of MnSOD on antioxidant capacity and inflammatory response in open heart surgery. In Vivo 22:147–151