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Comparison of Oxidative Damage and Antioxidant Parameters of Coronary Bypass Surgery Patients with Healthy Population

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Abstract *Aim:* The aim of our study was to investigate how effective the activities and levels of antioxidant parameters can be in the formation and balancing of damage by measuring them in patients for whom the decision to perform coronary revascularization operation was made. *Material and Methods:* Lipid peroxidation product malonyl dialdehyde (MDA) and superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) antioxidant parameters were compared as the parameters of oxidative damage in the preoperative blood samples of 78 patients (52 on-pump, 26 off-pump) undergone coronary bypass surgery and in the blood samples of healthy volunteer individuals. *Results:* A statistically significant increase was detected in the MDA levels of all CABG patients compared to healthy individuals (P=0.000). Similarly, the GSH level was significantly increased (P=0.000), while the CAT activity was decreased (P=0.000) in all CABG patients. Whereas, the SOD activity was found to be higher in the on-pump patient group in whom atherosclerosis was more advanced (P=0.01). *Discussion:* The increased blood levels of MDA in all CABG patient groups compared to the control group may be an indicator of the oxidative damage caused by advanced atherosclerosis. A significant GSH increase in all CABG patients with SOD levels in on-pump patients may be a balancing antioxidant activity for the damage. The low CAT levels in all CABG groups compared to healthy individuals might be associated with the development of atherosclerosis.
Conclusion: Atherosclerosis and oxidative damage were more advanced in CAD patients for whom the decision to undergo CABG surgery was made.

Keywords CABG, Catalase, Oxidative Damage, SOD

Introduction

Atherosclerosis is a major cause of death in developed countries [1]. Moreover, it is a chronic inflammatory disease characterized by the accumulation of lipid and inflammatory cells on the walls of medium and large arteries [2]. The pathogenesis of atherosclerosis involves the activation of pro-inflammatory signaling pathways, expression of cytokine/chemokine, and increased oxidative stress. Oxidative stress is an imbalance in favor of increased generation of reactive oxygen species (ROS) and/or body's reduced innate anti-oxidant defense systems [3]. Genetic susceptibility and environmental factors play a role in the pathogenesis. Most of these factors lead to endothelial dysfunction and other pro-atherogenic processes by causing oxidative stress [1]. ROS plays an important role in inflammatory response, apoptosis, cell growth and changes in the vascular tonus and also causes LDL-cholesterol oxidation, which also plays an important role in the atherogenesis [4].

SOD, glutathione peroxidases, catalases, paraoxonase, thioredoxins and nitric oxide (NO) are major antioxidant systems on the vessel wall. The production of ROS on the vessel wall increases in cases, such as hypertension, diabetes, smoking, and dyslipidemia, which are considered to be the risk factors for atherosclerotic cardiovascular disease (CVD) [5].

In our study, lipid peroxidation product malonyl dialdehyde (MDA) of the patients undergoing coronary artery bypass grafting (CABG) surgery was compared with that of healthy individuals as an indicator of preoperative oxidative damage, which is still widely perform worldwide. Besides a, by comparing some antioxidant parameters consisting of superoxide dismutase (SOD),catalase (CAT), and reduced glutathione (GSH) with that of healthy individuals, we aimed to determine the antioxidant activity in terms of formation and balancing of atherosclerosis.

Material and Methods

Fallowing the approval of the Fırat University Medical Faculty's Ethics Committee (Reference number: 2015-15) and after written informed consents were obtained from patients; total of 78 coronary artery disease (CAD) patients undergoing CABG surgery, 52 with cardiopulmonary bypass (CPB) (on-pump) and 26 with beating heart (off-pump) were included in this study. The study group consisted of all CAD patients who had been admitted to our clinic for elective coronary artery bypass grafting surgery (CABG).

The study was performed according to prospective study design and patient number was limited by coronary aretry patients operated in a single center by the same surgical team between April 2015 and July 2016. In the present study, we took blood samples from each patient before the surgical intervention (preoperative period). Emergency, chronic renal insufficiency and postoperative mortal patients excluded from study.

The control group consisted of allied health care staff, especially the physicians working in the hospital. After informing the individuals in the control group about the study, it was decided to take blood samples from those who volunteered to participate in the study by taking a short anamnesis and performing a physical examination. All samples were taken from upper extremity superficial venous system. In our control group consisting of healthy individuals, there were 3 individuals under the age of 20, 25 individuals aged 21-30 years, 38 individuals aged 31-40 years, 25 individuals aged 41-50 years, 7 individuals aged 51-60 years and 2 individuals aged 61-70 years. Of our control group, 6 had diabetes mellitus, 4 had hypertension and 35 were smoking.

Table 1: Descriptive Statistics for Characteristics of the Patients

Parameters	On-pump group (n:52)	Off-pump group (n:26)	Control group (n:100)
Mean age	63.42 years	69.47 years	37.97 years
Smoking n/%	12 (23%)	18 (69%)	35(35%)
Not Smoking n/%	40 (77%)	8 (31%)	65 (65%9
Diabetes Mellitus n/%	19 (37%)	6 (23%)	6 (6%)
Mean Coronary Graft	3.23	1.58	
Mild Peripherial Vascular Disease (ABI 0,9-07)	3 (6%)	2 (8%)	0
Advanced Peripherial Vascular Disease (ABI<0,7)	1(2%)	1 (4%)	0

In these blood samples, the biochemical parameters MDA, an oxidative damage indicator, GSH, CAT, and SOD, which are the antioxidant indicators, were analyzed.

All patients were managed by the same surgical and anesthetic team in the same operating room. Before the anesthesia induction, a radial artery catheter was inserted under local anesthesia and the preoperative blood samples were taken together with the baseline blood gas, and an invasive pressure monitoring was carried out as a standard procedure Membrane oxygenators and moderate systemic hypothermia were used to carry out CPB. In the off-pump patients, the operation was also performed using a stabilizer and intracoronary shunt. The off-pump operation was not performed on any of the patients requiring revascularization in the circumflex artery branches. After the operation, all patients were followed up in the intensive care unit.

Biochemical Analyses

Lipid Peroxidation

The determination of MDA in plasma was carried out based on the method of Placer et al. with slight modifications. MDA formed a pink complex with thiobarbituric acid (TBA) and the absorbance read was 532 nm. [6]. The plasma MDA content was expressed as nmol/ml.

GSH Level

The GSH level was determined in accordance with the method of Sarita et al.[7]. The GSH contents were expressed as µmol/ g Hb.

CAT Activity

The Aebi method was used to measure the CAT activity. The degradation rate of H_2O_2 by CAT was spectrophotometrically measured by means of H_2O_2 absorbing light at 240 nm wavelength [8]. The CAT activity was calculated as katal/g Hb.

SOD Activity

The SOD enzyme activity was measured based on the degradation of nitroblue tetrazolium (NBT) by the superoxide radical, which was produced by the xanthine-xanthine oxidase system. The blue Formosan obtained at the end of the reactions was maximally absorbed in 560 nm.[9]. The SOD enzyme activity was calculated as U/g Hb.

Statistical Analysis

The SPSS package software (15.0 for Windows) was used to carry out the statistical analysis. The unpaired t-test was used in the comparisons between all CABG patients, on-pump CABG patients in the 1st bypass and the 2nd bypass groups and peripheral patients and healthy individuals.

All results were shown as the mean±standart deviation. A p-value of <0.05 was considered statistically significant.

Results

When all CABG on-pump and off-pump (mean number of coronary grafts 3.23 and 1.58 respectively) patients were statistically compared with healthy individuals, it was determined that the MDA and GSH levels were significantly increased, while the CAT activity was significantly decreased in the patients than they were in healthy individuals. Moreover, when on-pump CABG patients were compared with healthy individuals, it was determined that the SOD enzyme activity was statistically significantly increased in the patients than they are in healthy individuals (Table 2, Table 3, Table 4).

Table 2: Comparison of the oxidative damage and antioxidant parameters of all CABG patients and healthy individuals

Parameters	N	Mean Control	Mean Patient	Mean±Std. Deviation	P
MDA nmol/ml	172	53.32	131.49	1.89±1.14	P≤0.000 (P=0.000)
GSH µmol/g Hb	176	53.23	132.81	5.26±8.45	P≤0.000 (P=0.000)
CAT k/g Hb	173	125.99	39.51	129.17±101.67	P≤0.000 (P=0.000)
SOD U/g Hb	177	83.83	95.56	33.97±7.04	P>0.05 (P=0.130)

Table 3: Comparison of the oxidative damage and antioxidant parameters of the on-pump CABG patients and healthy individuals

Parameters	N	Mean Control	Mean Patient	Mean±Std. Deviation	P
MDA nmol/ml	151	50.89	123.80	1.93±1.26	P≤0.000 (P=0.000)
GSH µmol/g Hb	150	53.10	117.72	3.40±6.27	P≤0.000 (P=0.000)
CAT k/g Hb	147	100.00	26.50	146.88±100.08	P≤0.000 (P=0.000)
SOD U/g Hb	151	69.39	88.58	34.06±6.55	P<0.05 (P=0.010)

Table 4: Comparison of the oxidative damage and antioxidant parameters of the off-pump CABG patients and healthy individuals

Parameters	N	Mean Control	Mean Patient	Mean±Std. Deviation	P
MDA nmol/ml	125	52.43	103.25	1.37±0.60	P≤0.000 (P=0.000)
GSH µmol/g Hb	124	49.64	110.98	4.08±7.95	P≤0.000 (P=0.000)
CAT k/g Hb	121	73.99	13.54	173.16±90.71	P≤0.000 (P=0.000)

SOD U/g Hb	125 64.43	57.54	33.18±6.51	P>0.05 (P=0.388)

Table 5: The demographic findings of all CABG patients

Σn 78	**Groups**	**Biochemical value (X±Sx)**					
		Urea (41.19 ± 3.92) P	**Creatinine (0.89 ± 0.01) P**	**AST (25.59± 3.48) P**	**ALT (27.99 ± 3.12) P**	**CRP (1.11 ± 0.27) P**	**% EF (0.49 ± 0.03) P**
Gender	Female	0.337	0.997	0.355	0.697	0.527	0.682
	Male	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
LIMA	Yes	0.757	0.472	0.834	0.753	0.392	0.197
	No	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
DM	Yes	0.672	0.972	0.164	0.278	0.352	0.092
	No	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
HT	Yes	0.252	0.917	0.614	0.742	0.291	0.149
	No	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Smoking	Smoking	0.612	0.981	0.520	0.811	0.633	0.673
	Non-smoking	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

Table 6: The demographic data of the on-pump patients

Σn 52	**Groups**	**Biochemical value (X±Sx)**				
		Urea	**Creatinine**	**AST**	**ALT**	**CRP**
Lima Presence	Yes	28.70±3.37	0.89±0.10	20.55±3.51	24.18±3.31	7.66±0.56
	No	38.22±4.57	0.85±0.50	18.92±3.11	22.37±3.87	7.20±0.52
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
DM Presence	Yes	40.56±4.32	0.99±0.05	20.00±3.53	20.77±4.52	7.83±0.53
	No	32.38±2.66	0.87±0.03	20.82±2.58	25.02±3.84	7.72±0.67
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
HT Presence	Yes	36.13±3.22	0.97±0.52	21.33±2.87	22.65±2.99	7.82±0.55
	No	30.43±4.36	0.96±0.35	20.18±3.12	24.28±9.46	8.23±1.24
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Smoking Status	Smoking	31.44±3.98	1.12±0.54	24.64±3.01	25.77±4.27	7.37±0.66
	Non-smoking	35.24±2.85	0.97±0.63	23.77±1.98	22.58±2.04	7.35±0.48
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
COPD Presence	Yes	42.65±5.65	1.04±0.77	20.10±3.87	25.38±4.87	5.49±0.65
	No	37.72±1.11	0.89±0.65	23.27±1.45	25.15±1.05	6.87±0.48
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

Table 7: The demographic data of the off-pump patients

Σn 26	**Groups**	**Biochemical value (X±Sx)**				
		Urea (31.30 ± 2.21) (39.47 ± 2.45) P	**Creatinine** (0.53 ± 0.19) (0.87 ± 0.17) P	**AST** (18.28 ± 1.87) (21.42 ± 2.89) P	**ALT** (18.37± 1.57) (23.28± 2.82) P	**CRP** (9.57 ± 2.25) (8.25 ± 1.32) P
DM Presence	Yes	0.127	0.221	0.193	0.226	0.674
	No	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
HT Presence	Yes	0.557	0.487	0.079	0.492	0.319
	No	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Smoking Status	Smoking	0.699	0.856	0.089	0.147	0.445
	Non-smoking	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
COPD Presence	Yes	0.049	0.134	0.262	0.649	0.805
	No	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

Discussion

Oxidative stress-induced excess ROS production is shown as the ultimate and critical mechanism in the development of atherosclerosis. ROS are a group of small reactive molecules that play critical roles in the regulation of various cell functions and biological processes. Uncontrolled ROS production plays a role in vascular damage [10]. The initial event during the development of atherosclerosis is the endothelial injury. This causes infiltration and accumulation of low-density lipoprotein cholesterol (LDL) in the subendothelial space. LDL gets oxidized to form oxidized LDL in pathologic states [11]. In our study, the levels of lipid peroxidation product MDA, which is a parameter of increased oxidative damage, were measured. The MDA levels were found to be significantly higher in all patient groups ($p \leq 0.000$) than they were in healthy individuals. We are of the opinion that coronary atherosclerosis might be effective on this significant increase in the MDA levels of patients for whom the decision to undergo CABG was made.

It has been found that three distinct types of SOD isoforms were expressed in mammalian tissues [12]. SOD1 (copper / zinc-SOD) is found in the cytoplasm and mitochondrial intermembrane space [13]. SOD2 is found in the mitochondrial matrix [14]. SOD3 is found in the extracellular matrix, on the cell surface, and in the extracellular fluids [15]. All three isoenzymes serve key antioxidant functions by catalyzing the dismutation of superoxide into oxygen and hydrogen peroxide [16]. It is proposed that the effects of SOD on atherogenesis are dose-dependent [17]. A moderate SOD1 upregulation decreases the ROS burden, while over-increased SOD activity can expand the oxidative damage by increasing the distal antioxidant activity. SOD1 overexpression generates a high amount of hydrogen peroxide, which can lead to the formation of proatherogenic molecules, such as hydroxyl radicals or metal-associated reactive species [18]. SOD2 is the first defensive enzyme against superoxide, the byproduct of the mitochondrial transport chain. Homozygous SOD2 mutant mice die within the first 10 days of life, indicating the importance of this enzyme [19]. The SOD3 isoform is intensely expressed on the vascular wall and its functional importance in the development of atherosclerosis has not yet been clarified. Considering the SOD activity in our study, there was no difference between the control group and all and off-pump CABG patients, while the SOD activity was determined to be significantly increased in the on-pump CABG patients with more advanced coronary artery involvement considering the mean number of grafts (3.23 versus 1.58). Although the isoforms of SOD enzyme were not specified in this study, the elevation in the on-pump group may be a cause of common atherosclerosis development, while the fact that the increase in the SOD activity was not so high suggests that it was rather effective to stabilize the oxidative damage in the formation of atherosclerosis. This finding of ours is also in parallel with previous studies [18,19].

GSH is a tripeptide thiol found in all cells. It has undertaken an important protective role against free radicals and reactive oxygen species in various tissues [20]. The decrease in the GSH levels in the endothelial cells results in more susceptibility to the toxic effects of reactive oxygen species [21]. In the study by Tsan et al., it was reported that the GSH concentrations increased by exogenous administration protected the endothelial cells against H_2O_2 induced injury [22]. Increased GSH levels in all groups are statistically significant as a stabilizing factor in patients with relatively advanced atherosclerosis decided to undergo a surgery, and are parallel to other study results.

CAT convert hydrogen peroxide into water and oxygen. They are found in peroxisomes and improve atherosclerosis in high-fat diet mice models [23]. Based on the type of atherosclerotic model studied; different results of antioxidant enzymes on the development of atherosclerosis are observed. In Apo-E KO mice, it is thought that atherogenic stimulation mostly develops as a result of the accumulation of peroxides. This state of peroxide accumulation is ameliorated, at least in part, by catalases [10]. Over-expression of catalase downgrade atherosclerosis in Apo-E KO mice [23]. Furthermore, in this mouse model, SOD1 overexpression was ineffective, while catalase downgraded atherosclerosis when a high-fat diet was given [23,24]. Considering our study, the fact that the p-value of the CAT enzyme activity ($p \leq 0.000$) was significantly lower in all patient groups compared to healthy individuals suggested that it played an active role in the development of common atherosclerotic coronary artery disease. However, it could not be understood whether the cause of this decrease was genetic or acquired.

Conclusion

Atherosclerosis and oxidative damage were more advanced in CAD patients for whom the decision to undergo CABG surgery was made. Although the type of operation to be performed after this decision was made, surgical team and central factors were effective, diffuse involvement of coronary artery disease and cardiac function of the patient, as well as other comorbid factors of the patient, were important. In this study, oxidative damage caused by coronary atherosclerosis was demonstrated to be significantly higher in all CABG groups compared to healthy individuals. Considering antioxidant enzyme activities to stabilize this damage, GSH again came to the forefront among all groups. Although SOD is the first prominent enzyme in oxidative damage, it was shown to be significantly higher in the on-pump group in whom coronary atherosclerosis was relatively more advanced compared to healthy individuals. In this study, it was revealed that the most important enzyme was catalase. We are of the opinion that its low rates in all patient groups play an important role in the development of the disease. However, further studies are needed to determine whether the decrease in this enzyme level is due to genetic factors or acquired disease. Limitations of this study younger mean age, low ratios of DM volunteers of the control group. May be comparison of non-surgical coronary artery patients with CABG patients will be also valuable and we are planning to do this

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