

Oxidative stress and DNA damage during cardiopulmonary bypass

DNA damage during cardiopulmonary bypass

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Abstract

Aim: The aim of this study is to measure the oxidative stress index (OSI) and DNA damage in patients undergoing cardiopulmonary bypass (CPB) surgery due to coronary artery disease. **Material and Method:** 25 patients operated for CPB surgery due to coronary artery disease were included in the study. By collecting heparin containing blood from the patients at 5 different times (Before CPB, during pump inlet, during placing of cross clamp, during taking out of cross clamp and after CPB) DNA damage was studied by Comet Assay method from mononuclear leucocytes and OSI in plasma was studied. **Results:** At 5 different times plasma OSI value was found respectively as 4.92 ± 1.73 , 5.20 ± 2.69 , 5.70 ± 2.06 , 9.88 ± 4.88 , 9.57 ± 4.54 whereas Arbitrary unit (AU), DNA damage were determined respectively as 28.48 ± 11.30 , 29.04 ± 9.43 , 31.44 ± 11.74 , 33.92 ± 7.96 , 38.56 ± 6.20 AU and $p < 0.001$. **Discussion:** During CPB, OSI and DNA damage gradually increased and a positive meaningful correlation occurred between these two parameters ($r = 0.882$, $p = 0.048$). As CPB period extended, oxidative stress and DNA damage increased as well as TOS and OSI increased, or vice versa.

Keywords

Cardiopulmonary Bypass; CPB; Extracorporeal Circulation; Oxidative Stress; DNA damage.

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Introduction

During CPB, the patient's blood is in contact with non-physiological and non-endothelial surfaces such as cannula, tubing set, reservoir, antifoaming agents, filters, pumps, heat exchangers and oxygenators. This contact activates blood proteins and immune system cells [1]. As the contact surface increases, the damage in the blood contacting nonendothelial surface increases. The immune response starting during CPB may cause organ dysfunction, that effects postoperative mortality and morbidity [2]. In continuity of this response, oxidative stress [3] initiated by catecholamines, complement system, oscillated cytokines from activated neutrophils, free oxygen radicals released during ischemia-reperfusion period, endothelium damage, kallikrein cascade, endotoxine oscillation [4] and stimulated by factors such as systemic heparinization, nonpulsatile current, removal of blood stream by cross clamping, anaesthetic drugs, reperfusion, acute dilutional anaemia, perfusion pressure, stream changes and lungs being out of circulation, play an important role [5].

Intensive reactive oxygen types (ROS) production or antioxidant defence reduction causes oxidative stress by making structural and functional modifications in biomolecules [6]. Oxidative processes start a series of events that consequently cause cell death via decrease in mitochondrial energy capacity [7]. During CPB, perfusion system, low arterial pressure, embolism and surgical manipulation also cause ischemia [8] resulting in excessive energy phosphate consumption and intracellular Ca accumulation [2]. During ischemia, fatty acid oxidation disorders and creatine phosphate anaerobic metabolism become main energy source of the cell [9]. However creatine phosphate runs short quickly, and rapidly improved acidosis limits glycolysis as well. In addition to the effect of the extracorporeal circulation, the activation of proinflammatory mediators, is also stimulated. As a result, consisted free oxygen radicals damage the cell membrane and myocardium [10]. During the restoration of the heart in CPB, the heart is stopped temporarily by putting cross clamp on aorta and the myocardium is exposed to ischemia. After taking out the cross clamp (after ischemia), reoxygenation of tissues is provided by sudden reperfusion, where the free O₂ radicals cause a phenomenon named as ischemia-reperfusion damage [6, 11]. Free oxygen radicals have a property of reacting fast with proteins, phospholipids and thiols, their attacks on DNA cause mutations, even cell deaths [4, 7]. Various clinical, epidemiologic and experimental studies show that there is a relation between free radicals, lipid peroxidation and its products and DNA damage and carcinogenesis [12, 13]. CPB and the pathology of the subjects have importance at least as DNA damage.

DNA damage can be detected in many methods. The recently developed method "Single Cell Gel Electrophoresis" (SCGE) also named as "Comet Assay" or "Microgel Electrophoretic Technique", is sensitive, fast and cheap in obtaining the spiral fracture [14]. The easily used SCGE method, shows even low level of DNA damages, provides its analysis with only few cells, with different cell and tissue groups, does not require too many equipments and, its reliable results can be obtained economically and evaluated within just few hours [15].

Material and Method

25 patients who were operated (by on pump) in the Cardiovascular Surgery Department of Harran University Hospital or The State Hospital in Sanliurfa were included in our study and 5

tubes of blood were collected from each of them consecutively before CPB, during the pump inlet, at cross clamping, at cross clamp removal and after CPB. During CPB, roller pump was used and non-pulsatile perfusion was applied. Commercial crystalloid cardioplegia (Plegisol®, 4-9 ° C) and tube sets (Bicakçilar Extracorporeal Tubing Set-Turkey) were used during surgery. Patients were treated with 1200 ml of Ringer's lactate or isolitol-priming solution. Routine CPB procedures were performed during surgery.

Preparation of Samples and Studied Tests

Collected blood samples were transferred immediately to heparin containing vacutainers. DNA oxidation levels and the damage was analyzed with Comet Assay method after separating mononuclear leucocytes from fresh blood samples. Then the rest of blood sample was centrifuged at 3000 rpm and the plasma was separated out. The samples were kept frozen and stored at -80 °C until handling. Total oxidative Status (TOS), Total Antioxidant Status (TAS) and Oxidative Stress Index (OSI) were studied as a result of lipid peroxide, lipid hydroperoxide, myeloperoxidase, ceruloplasmin, total peroxide in the separated plasma.

DNA Damage Identification with Alkali Mono Cell Electrophoresis Method (Comet Assay)

Comet assay method is based on agarose gel loads of DNA chain exposed to electrical field in alkali setting and their carriage according to molecular size. In this measuring method, the processes show differences according to lab conditions. Tice et. al. suggested 8 steps of SCGE method as isolation of cells, preparation of slices, lysis, disintegration of DNA spiral, electrophoresis, neutralization, coloring and evaluation [16]. In Comet assay method mono cells are placed in agar gel. After lysis, while the damaged and broken DNA pieces form tail format since they move in electrical field at different degrees due to having different loads and molecular weights, free and undamaged DNAs do not form tail because they migrate together in electrical field.

Since damaged DNA cells were evaluated by visual detection method in our study, in the examination of the undamaged cells, there were circular cells, less dense at sides, luminary outlook with brightness in the middle. This outlook of cells is evaluated as nonmigration. If DNA damage starts to occur, smooth margined outlook becomes ragged edge outlook due to migration of DNA fractures out of the cell. Depending on the severity of the damage, there occurs a stretch from centre to the edge. This outlook is named as stretch or low migration. As the damage increases, the cells take the shape of a comet, that means high migration [17, 18]. The last stage is apoptosis in which stretching is directly proportional to the damage. Also fluorescence density at the tail is parallel to the degree of the damage. In this method, the damage was classified in 5 categories due to the degree of DNA migration. The length of migration shows difference according to quantity of fragments, DNA chain breakages and alkali-labile fields levels. The evaluation was done in five categories as undamaged DNA is evaluated as category 0, the ones having maximum damage are evaluated as category 4. (Figure 1). Totally 100 cells were evaluated from each slide. The results were calculated as arbitrary unit (AU) by considering maximum damage as 400.

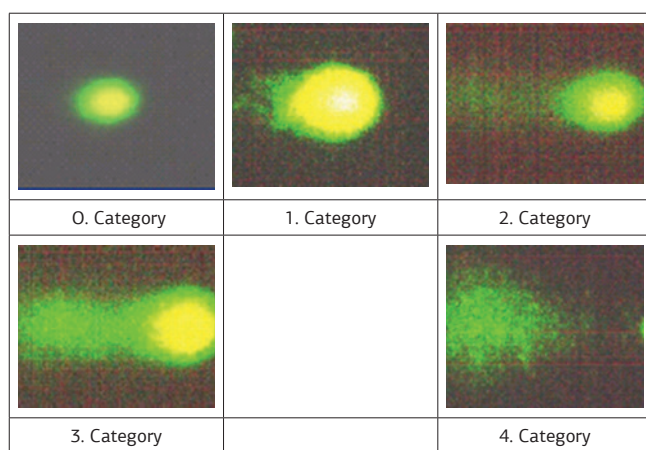


Figure 1. Images of DNA after electrophoresis migration of damage from oxidative DNA damage under the microscope.

Protein oxidation (PO)

15 µl plasma was mixed with 0,5 ml DNPH (10 milimolar 2,4-dinitrophenylhydrazine in 2 molar HCl) solution and left to incubation for 1 hour at room temperature. After incubation it was vortexed by adding 0,5 ml TCA (10% trichloroacetic acid in deionized water) solution and centrifuged for 3 minutes at 15000 rpm. After expelling supernatant, the pellet was washed 2 times with in the ratio of 1/1 ethanol/ ethyl acetate. 0,6 ml Guanidin HCl solution (6 molar guanidine HCl dissolutes in 20 milimolar pottsium phosphate (pH=2,3) was added and left to incubation for 15 minutes at 37 oC'de for dissolution. The absorbance of obtained colour was measured at 365 nm. After multiplying absorbance coefficient with (emax=22000/M/cm), the results were given as nmol (nmol/mgprot) per mg protein [17].

Plasma Lipide Peroxidation Measurement

25 µl plasma was mixed with 0,5 ml DETBA (10 mmol/L 1,3-diethylthiobarbituric acid, dissolved in 75 mol/L phosphate (pH=3)) and left in incubation for an hour at 96 oC. After leaving samples in ice bath for 5 minutes, 2,5 ml n-butanol was added. The mixture was vortexed and centrifuged at 1500 x g at 4 oC for 10 minutes. The supernatant was taken and analyzed in flormeter (Extantion= 539 and emission= 553)(Schimatzu, Japan) [19].

Total Antioxidant Status (TAS) Level Measurement

The measurement method, which uses Total Antioxidant Status Assay Kit (Rel Assay, Product Code: RL0017), is based on decolorization of all antioxidant molecules in the sample in proportion to the total concentration of antioxidant molecules of colourful radicals as a result of reduced colourful ABTS* cationic radical. Trolox, a water-soluable analogue of vitamine E is used as calibrator. The results were given as mmol Trolox Equivalent/L [20].

Total Oxidant Status (TOS) Level Measurement

In total automatic colormetric method, Total Oxidant Status Assay Kit (Rel Assay, Product Code: RL0024) is used. The colormetric method is based on oxidization of ferrous ions of oxidant molecules to ferric ion cumulatively.

The results are given as µmol H2O2 Equivalent/ L [21].

Oksidative Stress İndex (OSI)

OSI, as a sign of Oxidative Stress, is defined as percentage of the degree of TOS levels to TAS levels. In calculation of OSI

value of samples, TAS levels are multiplied with 10 and their units are balanced with TOS levels [24]. The results are defined as Arbitrary Units (AU).

$$OSI = \frac{TOS, \mu\text{mol H}_2\text{O}_2 \text{ Equiv. / L.}}{TAS, \mu\text{mol trolox Equiv. / L.}} \times 100$$

Statistical Analyses

Statistical analyses were done by using SPSS programme. First of all, normality evaluation was done with normality test "Shapiro-Wilk". In normal distribution, "Pearson" analysis was used for parametric correlations, while "Spearman" analysis was used for nonparametric correlations in abnormal distribution. p<0.05 value was considered significant.

Results

Demographic and characteristic data are given in Table 1. In the table, the gender (8 Male + 17 Female), average age (45.88 years), average height (161.52 cm), average weight (68.12 kg) and average BMI (1.71 kg/m²) values were given.

TAS, TOS, OSI and mononuclear leucyte DNA damage of patients are shown in Table 2.

1. Blood before pump, 2. Blood taken at the pump inlet, 3. Blood taken when a cross clamp is placed, 4. Blood taken after cross-clamp removal, 5. Blood taken from the pump

TAS level continuously reduces until the 4th blood sample (after removal cross clamp), (Table 1) and increases a little bit at the 5th blood (pump outlet) (Figure 2). TOS reduced a little as pass-

Table 1. Comparison of physical values of patients

	Patient (n = 25) Mean
Gender (M/ F)	8/17
Age (years)	45.88
Length (cm)	161.52
Weight (kg)	68.12
BMI (kg/m ²)	1.71

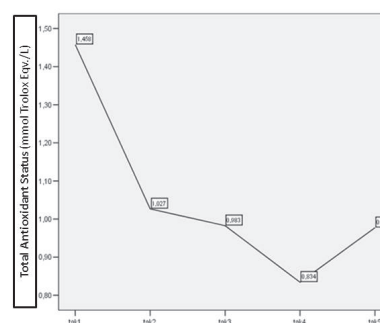


Figure 2. TAS exchange in plasma during CPB (mmol Trolox Eqv./L)

ing from the 1st blood sample (before pump) to the 2nd blood sample (at the pump inlet), then increased continuously after the 2nd blood (after pump inlet) (Figure 3). Until the 4th blood sample, OSI levels (as cross unclamping) continuously increases, at 5th blood sample there is a little reduction (Figure 4). This increase in OSI levels became maximum after unclamping. (4th blood sample). DNA damage on the other hand continu-

ously increased from 1st blood sample (before pump) to the 5th blood sample. (pump outlet) (Figure 5). Generally TOS, OSI, DNA damage increase while TAS level decreased.

According to the correlation analysis done at the end of study, a significant positive correlation was found ($r=0,882$ $p=0,048$) between OSI and DNA damage (Table 3). The distribution of relations between mononuclear leucocyte DNA damage and oxidative stress index levels were shown in Figure 6.

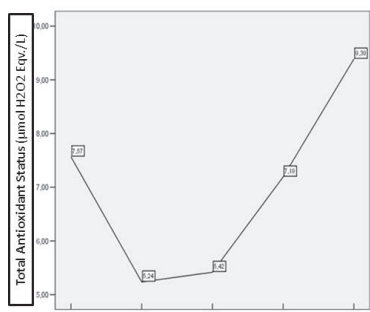


Figure 3. TOS exchange in plasma during CPB (µmol H2O2 Eqv./L)

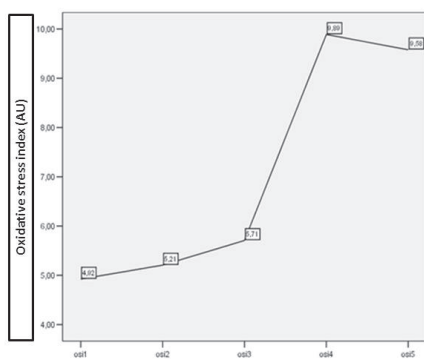


Figure 4. Oxidative stress index change in plasma during CPB (AU)

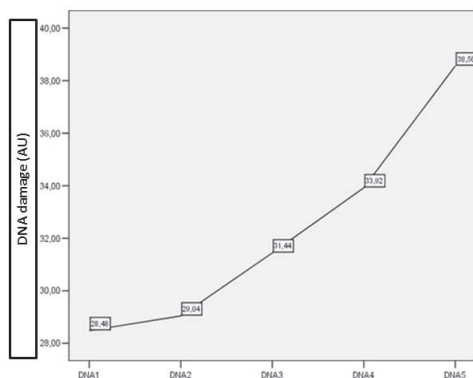


Figure 5. Change in DNA damage during CPB (AU)

Discussion

According to the results of our study, TAS continually decreased until unclamping the aorta, myocardium became bloodstained and TAS value increased after this period. This situation shows us that TAS value continuously decreased when there is no bloodstain, but TOS value continuously increased. This situa-

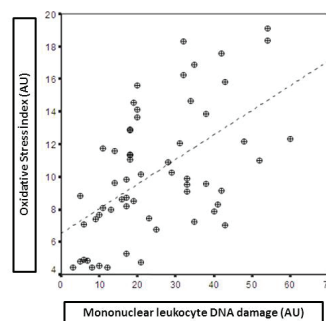


Figure 6. Distribution of the relationship between mononuclear leukocyte DNA damage and oxidative stress index levels during CPB.

Table 3. Relationships between DNA damage and Oxidant / Antioxidant parameters

		TOS	TAS	OSi
TAS	r	-0,058		
	p	0,926		
OSI	r	-0,330	-0,661	
	p	0,587	0,224	
DNA	r	-0,335	-0,576	0,882
	p	0,581	0,309	0,048(*)

* p < 0.05

tion shows us that during CPB increases oxidative stress continuously due to either unfamiliar surface material or surgical situations. Besides, the antioxidant defence systems become insufficient due to the decrease of TAS, but the increase of TOS value. In the analysis, TAS and TOS values decreased a little during the pump inlet because of the hemodilution. OSI levels continuously increased at cross clamping and was maximum at unclamping. When the circulation of the heart stopped during the cross clamping period, oxygen derivative radicals are generated from ischemi reperfusion as a result of bloodstained heart and increase OSI levels. In other words, oxygen derivative radicals cause oxidative stress. Also due to the decrease of antioxidant level during pump period, oxidant level and oxidative stress cannot be eliminated, accordingly OSI continuously increases. Due to the increase of TOS and OSI levels and the decrease in TAS levels, DNA damage continuously increased, because of non endothelial level, non pulsatile current, insufficient perfusion, discontinuation of blood stream with cross clamping, anesthetic drugs, myocardium damage, cells in stress due to reperfusion and immune response and attacks of oxidative stress elements to DNA, during CPB. Our study shows that OSI and DNA damage continuously increased during the pump and a positive correlation between these two parameters occurred. As the length of clamping period and pump period extended, oxidative stress increased and more DNA damage occurred. Many studies show that ROS levels can severely increase in stress periods and cause serious damage in many molecular molecules such as lipides, proteins and DNA.

The results of our study are parallel to many previous studies. Starkopf et. al. stated that TAS decreases and oxidant damage occurs during ischemia and reperfusion period in CBP. Also, in coronary artery bypass facts during 72 hours after surgery, it was observed that TAS was depressed, lipid peroxidation increased and depression in TAS were in reverse relation with

Table 2. Comparison of DNA damage and Oxidant / Antioxidant parameters of patients

Parametreler	1.Blood	2. Blood	3. Blood	4. Blood	5. Blood
TAS (mmol Trolox Eqv./L)	1.45 ± 0.41	1.02 ± 0.25	0.98 ± 0.33	0.83 ± 0.29	0.97 ± 0.38
TOS (µmol H ₂ O ₂ Eqv./L)	7.56 ± 4.17	5.23 ± 3.54	5.41 ± 2.32	7.19 ± 3.35	9.38 ± 5.92
OSI (AU)	4.92 ± 1.73	5.20 ± 2.69	5.70 ± 2.06	9.88 ± 4.88	9.57 ± 4.54
DNA (AU)	28.48 ± 11.30	29.04 ± 9.43	31.44 ± 11.74	33.92 ± 7.96	38.56 ± 6.20

lipid peroxidation (23 tubing set, reservoir, antifoaming agents). According to the study of Taşkıran A. et. al. on the damage and relation of plasma TAS levels with ischemia-reperfusion damage before CPB coronary artery bypass surgery, they found out that low TAS values before surgery are in relation with ischemia-reperfusion damage and severity of myocardium damage [24, 25]. Ischemia and reperfusion damage during CPB cause proinflammatory mediators and ROS formation by causing an important myocardial stress, which harms proteins, lipides that affect postoperative cardiac function as well as DNA. The existence of positive correlation between DNA damage and oxidants and negative correlation between DNA damage and antioxidants, force to find better methods that can reduce side effects of CPB to minimum level. As a result, factors such as contact with artificial surfaces, surgical trauma, oxidative stress and ischemia reperfusion injury play an important role in the formation of DNA damage. As a result of these factors, reactions at the molecular level increase DNA damage during CPB. Among the limitations of this study is the absence of a control group from patients operated by non-pump techniques.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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