OXIDATIVE STRESS AND INFLAMMATION ARE INCREASED IN FIRST FIVE DAYS IN CORONARY ARTERY BYPASS SURGERY PATIENTS: A PROSPECTIVE STUDY

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[Aumento dello stress ossidativo ed infiammazione nei primi cinque giorni in bypass coronarico chirurgia pazienti: uno studio prospettico]

ABSTRACT

***Background and aim**: The aim of this study was to investigate the alterations in oxidative stress, antioxidant status and inflammation in first five postoperative days after coronary artery bypass surgery (CABG).*

***Material and method**: Thirty two patients scheduled for CABG were recruited. Blood samples in preoperative and postoperative 4th, 24th hours and 3th, 5th days have been collected and examined for lipid hydroperoxite (LOOH), catalase, free sulphydril (-SH), and paraxonase, arylesterase and neopterin levels.*

***Results**: Paraoxonase (preoperative 132.3±67.8 vs. postoperative 5th day 108.2±47.2 p<0.05), arylesterase (preoperative 113.2±29.9 vs. postoperative 5th day 94.3±44.1 p<0.05) and anti-oxidant parameters [-SH (preoperative 0.18±0.03 vs. postoperative 5th day 0.17±0.03 p<0.05) and catalase levels (preoperative 13.9±3.3 vs. postoperative 5th day 9.7±4.4 p<0.05)] activities were significantly decreased. –LOOH levels (preoperative 8.4±2.6 vs. postoperative 5th day 11.4±3.3 p<0.05) and neopterin levels (preoperative 7.2±5.2 vs. postoperative 5th day 12.9±9.0 p<0.05) were significantly increased on the 5th postoperative day compared to preoperative levels.*

***Conclusion**: Inflammation and oxidative stress in CABG patients were found to be altered on the postoperative 5th day that should alert clinicians for possible complications.*

Key words: Coronary artery bypass, oxidative stress, antioxidant, inflammation.

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Introduction

Coronary artery bypass surgery (CABG) associated morbidity and mortality is influenced by several events such as surgical trauma, ischemia-reperfusion and patient characteristics[1]. An increasing number of patients presenting for cardiac surgery are elderly, with a high incidence of co-morbidities. Advanced age, significant co-morbidities and the procedure - cardiopulmonary bypass (CPB)- are associated with an enhanced degree of oxidative stress[2] and clinically with higher rates of postoperative complications and an increased length of hospital stay[3-5]. Formation of reactive oxygen species (ROS), during postischemic reperfusion, triggers a complex chain of events. The nature of these oxidative events leads to depletion of plasma antioxidants, increased lipid peroxidation, and formation of other detrimental metabolites[1,6].

In the recent years, several clinical studies pointed-out that plasma levels of neopterin, a well known marker of inflamation, is significantly high in patients with various cardiovascular diseases (e.g. coronary artery disease, unstable angina, acute coronary syndrome and myocardial infarction)[7-8].

The CPB-induced inflammatory response and oxidative stress could result in myocardial injury and a variable degree of myocardial dysfunction after surgery under CPB[9-10].

It is still a debate t the duration of the postoperative alterations in oxidative stress and inflammations after CABG. There is, to our knowledge, no research about these parameters in patients undergoing CABG.

Accordingly, we aimed to investigate paraoxonase, arylesterase activities, lipid hydroperoxide (LOOH), free sulfhydryl groups (–SH = total thiol), catalase and neopterin levels during the first five postoperative days in CABG patients.

Material and method

Study population

This study was conducted at the Departments of Cardiovascular Surgery and Clinical Biochemistry of Harran University, from February 2009 to May 2011. The study protocol was approved by the Ethics Committee of Medical School, of Harran University Şanlıurfa, Turkey. The study group consisted of 32 consecutive patients who were hospitalized for elective CABG. Written informed consent was obtained from all subjects entering this study.

Exclusion criteria

Exclusion criteria were the presence of neoplastic diseases, concomitant inflammatory diseases such as infections and auto-immune disorders, chronic obstructive pulmonary disease, major depression, hepatic and renal disorders, valve repairs or other cardiac procedures, patients with left ventricular ejection fraction <30%, emergency CABG and recent major surgical procedure.

Anesthetic technique

Anesthesia consisted of a balanced opiate-based general anesthesia technique. Induction was performed by infusion of propofol (1.5 mg/kg), rocuronium (1 mg/kg) and fentanyl (1μg/kg). Anesthesia was maintained with sevoflurane as an inhalation agent according to desired bispectral index (BIS) values (BIS values 40-65). Hypertension was treated with vasodilators (nitroglycerin and nitroprusside). A mean arterial pressure of 60 mmHg or higher and a heart rate less than 70 beats per min was maintained. Heparin was administered at 300 IU/kg for the CABG group. After all anastomoses were completed, heparin was neutralized with protamine sulfate.

All patients received standardized postoperative care. Tracheal extubation is accomplished when the patient was hemodynamically stable, responsive and cooperative, FiO2, 50% PaO2 >11 kPa, pH 7.3, core temperature 36.8 C° and without excessive chest tube drainage. Postoperative pain relief was achieved with intravenous morphine (0.5 mg kg/h) and paracetamol, 1000 mg administered three times daily.

Surgical techniques

The operations were performed under general anesthesia by two cardiac surgeons. CPB was instituted with an ascending aortic cannula and a two stage right-atrial cannula. Internal mammary artery, saphenous vein and radial artery grafts were used in patients. Distal and proximal graft anastomoses were performed using a single cross-clamp technique. Myocardial protection was achieved using antegrade and/or retrograde isothermic blood cardioplegia. Mild systemic hypothermia with a core temperature of 32°C was maintained during CPB.

Blood sample collection

Blood samples were drawn for biochemical analysis from the cubital vein at 12 hour before operation, postoperative 4th hour, 1st, 3rd and 5th days. The blood samples were kept at room temperature for 30 min and then separated from the cells by centrifugation at 3000 rpm for 5 min. Serum samples were stored at –80 °C until the time of biochemical analysis. Blood samples were obtained following an overnight fasting state.

Measurement of paraoxonase and arylesterase activities

Measurements of paraoxonase activities were performed in the absence basal activity. The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was measured by monitoring the increase of absorbency at 412 nm at 37°C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH:8, which was 17 000 M - 1 Cm - 1. Paraoxonase activity was expressed as U L- 1 serum. Phenylacetate was used as a substrate to measure the arylesterase activity. Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol; 1310 M 1 Cm - 1. One unit of arylesterase activity was defined as 1 µ mol phenol generated min - 1 under the above conditions and expressed as U L Serum.

Measurement of serum lipid hydroperoxide

Serum lipid hydroperoxide (LOOH) levels were determined by the ferrous ion oxidation–xylenol orange (FOX-2) method as previously described. The method is based on a known principle of the oxidation of Fe II to Fe III by lipid hydroperoxides, under acidic conditions.

Measurement of total free sulfhydryl groups of serum samples

Free sulfhydryl groups (–SH) of serum samples were assayed according to the method of Ellman[23] as modified by Hu et al[24]. Briefly, 1 mL of buffer containing 0·1 M Tris, 10 mM EDTA, pH 8.2 and 50 L serum was added to cuvettes, followed by 50 L of 10 mM DTNB in methanol. Blanks were run for each sample as a test, but there was no DTNB in the methanol. Following incubation for 15 min at room temperature, sample absorbance was read at 412 nm on a Cecil 3000 spectrophotometer (Cecil Instruments, Cambridge, UK). Sample and reagent blanks were subtracted. The concentration of sulfhydryl groups was calculated using reduced glutathione as the free sulfhydryl group standard and the results were expressed as millimolars.

Measurement of neopterin

The serum NP concentrations were measured by commercial ELISA kit (Quantikine; R&D Systems, Vienna, Austria) in duplicate. According to the manufacturer's information, sensitivity of the assay was 10nmol/L. The intra-assay variability coefficients were 1.1–3.1%, while the inter-assay variability coefficients were 5.6– 6.1%. The investigator performing the assay was blinded to all clinical data and outcomes.

Statistical analysis

All data analysis were conducted using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA), with continuous parameters expressed as means ± standard deviations. Distribution of continuous variables was analyzed with the one-sample Kolmogorov–Smirnov test, and all data were distributed normally. One way analysis of variance (ANOVA) with post hoc Bonferroni test was used for continuous variables.. Power calculations based on a pilot study with 12 patients to detect a significant difference in paraoxanose levels ($\alpha = 0.05$, power = 0.80) indicated that 32 patients were needed. Two-tailed $p<0.05$ was considered as statistically significant.

Results

In this study we evaluated 32 patients with ages ranging between 37 and 78 years (62±9.59). There were 14 female (43.8%) and 18 male (56.2%) patients. Nine patients were diabetic, 19 patients were hypertensive, 17 patients were smoker and 12 patients were dislipidemic. The demographic data and data on CABG were summarized in Table 1.

Male [n (%)]	18 (%56.2)
Female[n (%)]	14 (%43.8)
Age (year)	62±9.59 (37-78)
Cardiovascular Risc Factor	
Hypertension [n (%)]	19 (% 59.4)
DM [n (%)]	9 (% 28.1)
Dyslipidemia [n (%)]	12 (% 37.5)
Smoking [n (%)]	17 (%53.1)
COPD [n (%)]	9 (%28.1)
PAD [n (%)]	3 (%9.4)
CCS	2.09±0.46 (1-4)
Preop MI [n (%)]	15 (46.8)
Ejection Fraction (%)	45.26±8.58 (25-70)
Graft (n)	2.27±0.6 (1-4)
CCT (min)	86.7±31.1 (40-125)
CPBT (min)	132.3±44.6 (46-175)
ICU stay (day)	2.31±1.51 (1-9)
Hospital stay (day)	10.4±5.21 (1-27)

Table 1: General characteristics of patients.

DM: Diabetes Mellitus COPD: Chronic Obstructive Pulmonary Diseaseı PAD: Peripheral Artery Disease CCS: Canadian Coronary Score MI: Myocardial Infarction CABG: Coronary Artery Bypass Grafting CCT: Cross Clamp Time CPBT: Cardiopulmonary Bypass time ICU:Intensive Care Unit

Results of serum LOOH, catalase, -SH, paraxonase, arylesterase and neopterin levels at preoperative, postoperative 4th, 24th hour, postoperative 3th, 5th day were shown on Table 2. Paraxonase levels were statistically significantly decreased at postoperative 5th day compared to preoperative levels ($p<0.05$). -SH and catalase levels were statistically significantlty decreased at postoperative 4th, 24th hour, postoperative 3th, 5th day compared to preoperative levels ($p<0.05$ for all comparisons). –LOOH and Neopterin levels

	Preoperative	Postoperative 4th hour	Postoperative 1st day	Postoperative 3rd day	Postoperative 5th day	P value (Anova)
Arylesterase (U/L)	113.2±29.9	97.0±35.3*	105.1±47.2	100.1±45.5	94.3±44.1*	p<0.001
Paraoxanase(U/L)	132.3±67.8	127.3±56.5	124.9±48.9	122.2±49.7	108.2±47.2*	p<0.001
Catalase (U/L)	13.9±3.3	7.0±4.1*	8.3±3.3*	8.9±4.5*	9.7±4.4*	p<0.001
Neopterin(nmol/L)	7.2±5.2	9.3+5.4	10.2+6.5*	15.0±10.1*	12.9±9.0*	p<0.01
-SH (mmol/L)	0.18±0.03	0.2±0.04	0.17±0.02*	0.16±0.01*	0.17±0.03*	p<0.01
-LOOH (µmol/L)	8.4±2.6	7.7±1.0	10.4±3.1*	11.7±3.3*	11.4±3.3*	p<0.01

Table 2: Levels of enzyme
**:p<0.05 when compared to preoperative level*

were statistically significantly increased at postoperative 24th hour, postoperative 3th, 5th day compared to preoperative levels (p<0.05 for all compari-sons).

Discussion

With the present study we pointed-out that:

• paraoxonase and arylesterase activities,

• and anti-oxidant parameters [-SH and catalase levels] were significantly decreased whereas,

• LOOH

• and neopterin levels were significantly increased in the first five postoperative days after CABG. This is the first study evaluating LOOH and –SH, paroxanase, arylesterase, catalase and neopterin levels in the first 5 postoperative days in CABG patients.

Cardiac surgery induces oxidative stress and systemic inflammatory response (SIR) by activating plasma proteins (complement, coagulation, and fibrinolysis) and blood cells (neutrophils, monocytes/macrophages, and lymphocytes). Extracorporeal circulation (EC) of blood during cardiopulmonary bypass also has been shown to induce the production of several pro-inflammatory molecules. The ensuing systemic inflammatory response and the superimposed period of ischemia-reperfusion are conditions that promote the production of oxygen-derived free radical species, which are able to initiate lipid peroxidation and a chain of events leading to cell membrane damage, tissue injury, and functional impairment[11]. If this response is exaggerated, it may lead to substantial morbidity, including myocardial dysfunction, respiratory failure, renal and neurological dysfunction, bleeding disorders, altered liver function, and, finally, increased mortality[12]. Increased LOOH levels after CABG supports previous studies revealing increased oxidative stress[11-12] and increased LOOH[13] in CABG patients.

Besides increased oxidative stress, deranged antioxidative mechanisms might play role in CABG induced detrimental effects as is evidenced, in the present study, by significantly decreased –SH and catalase levels, supporting previous study of Akila et al whom revealed significantly decreased G-SH levels in both on-pump and off-pump CABG patients[14]. Similarly we have revealed significantly decreased PON1 and arylesterase activities in patients after CABG, which supports previous reports revealing decreased PON1 activity was lower in subjects with CAD and there was a significant relationship between PON1 activity and the severity of CAD[15-18], as well as it could be noticed a significant PON 1 activity reduction after CABG operation[19]. There are several studies about the role of NP –marker of cell-mediated immunity([20]) after cardiac surgery[21-24], revealing that levels of NP at 24 and 48 hours after surgery is a good predictor of mortality, of sepsis and postoperative complications secondary to CPB[21]. Moreover, preoperative NP levels higher than 10 nmol/L were proved to be related with prolonged ICU stay[25], and increased NP concentrations after cardiac surgery are accused to be related to endothelial damage[24]. Increased postoperative NP levels until post-operative 5th day supports previous studies assessing NP levels in subjects undergoing CABG[21-25].

Several limitations of the study should be noted. We included 32 patients with coronary artery bypass surgery. Although we conducted a power analysis according to our pilot study results, our sample size might be small and this study was an observational study and adding control group to study design might add to the value of the findings of this study. It should be noted that generalizing data of the present study might not be appropriate because our results denote therapeutic responses of a population from the same geographic region and genetic origin; subjects from other geographic region(s) and genetic origin(s) might respond differently to study medications than did our study population.

In conclusion, increased LOOH, NP levels besides decreased PON1 and arylesterase activities and decreased serum –SH levels up to 5th postoperative day in subjects undergoing CABG suggests ongoing inflammatory response and alterations in oxidative stress and antioxidant status which necessitates vigilant clinical observation after CABG to prevent postoperative complications.

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