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Lipid Peroxidation Product and Glutathione Levels in Patients with Coronary Heart Disease Before and After Surgery

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Abstract

Background: It has been reported that ischemia-reperfusion is associated with augmentation of oxidative stress and its specific and sensitive markers. Oxidative stress may cause atrial fibrillation (AF) which is a common consequence after cardiac surgery. Dietary supplementation with antioxidants might lower the incidence of AF following coronary artery bypass graft (CABG) surgery. **Materials and Methods:** Fifty patients with coronary heart disease (CHD) referred to Namazi and Faghihi Hospitals in Shiraz, undergone elective CABG, were enrolled in this study. For evaluation of oxidative stress, whole blood was taken before and 24 hours after surgery and malondialdehyde (MDA) as an oxidative marker and glutathione (GSH) as an antioxidant marker were measured. **Results:** Results showed a significant difference between the mean concentration of GSH before and after CABG surgery ($P < 0.05$); however, the difference in plasma MDA levels before and after CABG was insignificant. **Conclusion:** CABG surgery results in oxidative stress and reduces GSH 24h after surgery and administration of antioxidants may attenuate post-operative oxidative stress. [GMJ.2015;4(2):78-82]

Keywords: Coronary Artery Bypass Grafting; Oxidative Stress; Malondialdehyde; Glutathione; Lipid Peroxidation

Introduction

Oxidative stress has a critical role in pathophysiology of atherosclerosis and acute thrombotic events including dyslipidemia leading to oxidation of low density lipoprotein (LDL), atheroma formation, plaque rupture and recurrent thrombosis. Moreno and Fuster showed the relationship between various coronary diseases risk factors like elevated blood pressure, diabetes and cigarette smoking with atrial diseases [1]. Atrial fibril-

lation (AF) is a common complication of cardiac surgery because of electrophysiological and structural changes of coronary heart arteries [2]. Incidence of AF after coronary artery bypass grafting (CABG) surgery is dependent on age, severity of cardiac disease, case complexity and co-morbidities. AF can increase complications such as strokes, co-medication consumption, duration of admission and mortality in hospital and also can reduce survival after surgery [3]. Main factors leading to AF are cardiac overload initiating atrial elec-

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trophysiological remodeling and inflammation, starting a second messenger system and cause oxidative stress [4, 5]. After transient ischemia, the tissue releases reactive oxygen species (ROS) which are specific and sensitive markers for inflammation and oxidative stress. ROS release in reperfused myocardium may be used as a method for comparing inflammatory process in CABG. [6,7].

In addition to beta-blockers, several pharmacologic medications are reported for the prevention of AF such as ascorbic acid and other antioxidants [8-10].

In this study, we aimed to compare lipid peroxidation product malondialdehyde (MDA) levels and glutathione (GSH) levels in patients with coronary heart disease before and 24h after CABG surgery.

Materials and Methods

Materials

All chemicals used in this study were analytical grade and were obtained from Sigma (Sweden) and Merck (Germany).

Subjects

Fifty patients with CHD participated in this study. The study was approved and performed under the guidelines of Ethics Committee of Shiraz University of Medical Sciences. Informed consent was obtained from each subject before blood sampling.

Fifty patients (30 men and 20 women) with CHD referred to Namazi and Faghihi Hospitals were enrolled in this study. The inclusion criteria included patients with atherosclerotic triple vessel disease nominated for elective CABG without major surgical risk factors or abnormal biochemical investigations. All patients aged 40 to 50 years old with negative history of antioxidant supplementation consumption and their weight were 75 ± 5 kg. Patients with previous history of rheumatoid arthritis, asthma and autoimmune diseases were excluded from the study.

For evaluation of oxidative stress, 3ml whole blood was taken before and 24 hours after surgery, MDA was measured by thiobarbituric (TBA) method and GSH was evaluated by colorimetric method.

Sample Processing

Blood samples were drawn into EDTA anticoagulant tubes and were centrifuged within 4 hrs after sampling. Plasma samples were immediately frozen and kept at -20°C for analysis.

Measurement of Plasma Total Glutathione

The assay of GSH with 5-5'-dithiobis[2-nitrobenzoic acid] (DTNB) was performed and followed by a standard Ellman's method [11]. The standard curve was generated using a 1mM solution of GSH. Clear plasma was analyzed for GSH levels. Moreover, 2.3ml of potassium phosphate buffer (0.2 M, pH 7.6) was added to 0.2ml of plasma samples, followed by the addition of 0.5 ml DTNB (0.001 M) in a buffer. The absorbance of reaction products was observed after 5min at 412nm.

Estimation of Plasma Lipid Peroxides

MDA, an indirect index of lipid peroxidation, was assayed as thiobarbituric acid reactive substances (TBARS) using a colorimetric method [12]. Briefly, 0.5mL of plasma was added to 2mL of TBA reagent containing 0.375% TBA, 15% trichloroacetic acid and 0.25mol/L HCl. The mixture was boiled for 15min, cooled and centrifuged at 1700g for 15min at 4°C . The absorbance of supernatant was measured at 532nm. TBARS concentration was calculated using 1,1,3,3-tetramethoxypropane as a standard. Results are expressed as nanomoles per milliliter of plasma.

Statistical Analysis

Statistical analysis was performed by paired T-Test, P-value < 0.05 was considered significant. All data were expressed as mean \pm standard error of the mean and analyzed using SPSS 16 software.

Results

Fifty patients (30 men and 20 women) with CHD referred to Namazi and Faghihi Hospitals were included in this study, their mean age was 45 ± 5.5 years and the patients' weight was 75 ± 5 Kg. The results showed a significant difference between mean concentration of GSH before (1192.32 $\mu\text{M/l}$) and af-

ter (1109.06 $\mu\text{mol/l}$) CABG surgery ($P < 0.05$); however, the difference in plasma MDA levels before (3.169 nmol/l) and after (3.164 nmol/l) CABG was insignificant (Figure. 1, 2).

Discussion

CABG surgery with cardiopulmonary bypass (CPB) and cardiac arrest induce an inflammatory response and reperfusion of this ischemic organ is the only way to limit ischemic damage but on the other hand, it leads to the generation of oxidative stress, which can cause post-ischemic tissue injury [13]. For lipid peroxidation parameter, MDA is a marker for oxidative stress and GSH is an excellent anti-

oxidant.

In this study, we demonstrate a significant difference between GSH levels in CHD patient plasmas before and after CABG surgery. This finding confirms the results of other investigations. Carlucci et al. showed that during ischemic period, a reduction in cellular NAD and GSH levels was seen. Besides, oxidative stress and endothelial damage are major events during CABG [14]. However, there are some results indicating that GSH level was decreased and catalase activity increased to similar values between on-pump or off-pump groups with little difference between them [15]. These observations may support the assumptions of Milei and his colleagues showing cardiac GSH release was negligible at baseline, but it increased 15 min after reperfusion; concomitantly, myocardial antioxidant, ubiquinol decreased. Also, neither coronary sinus blood nor cardiac biopsies showed increased lipid peroxide concentrations [16]. However, in critically ill patients, an intervention to attenuate oxidative stress might be considered beneficial, because reactive oxygen species may contribute to myocardial stunning, infarction and apoptosis and vascular dysfunction [17-19]. Altaei showed a lower concentration in GSH level and increasing MDA after CABG surgery, he also showed anti-inflammatory and antioxidant effects of silymarin treatment that provided protection against reperfusion injury and inflammation after CABG surgery [20].

Some papers demonstrated an increasing level of MDA in on-pump CABG group in comparison with preoperative levels [21, 22]. We did not get any significant differences in MDA concentration before and after CABG. Although Lazzarino et al. demonstrated that reperfusion ischemic myocardium during and after aortic cross clamping in coronary bypass surgery caused lipid peroxidation and increased formation of MDA in the myocardium of patients. However, they used HPLC for measuring MDA which is much specific for MDA as compared with TBA assay, which was used in this study. It has been shown that the assay does not detect real concentrations of MDA, because heating process may lead to decomposition of lipid hydroperoxides and

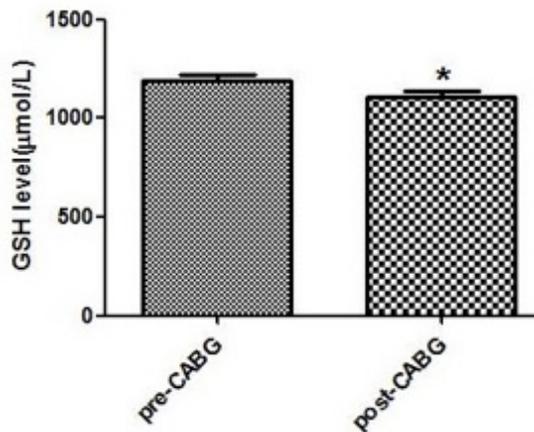


Figure 1. Plasma GSH (Mmol/L) in CHD Patients, Pre and Post CABG. Values Are Mean \pm SEM (N=50 in Each Group). $P < 0.05$.

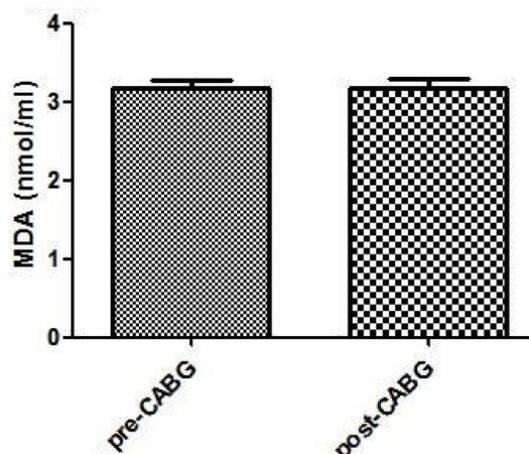


Figure 2. Plasma MDA (Nmol/mL) in CHD Patients, Pre and Post CABG. Values Are Mean \pm SEM (N=50 in Each Group). $P < 0.05$.

cycloperoxides. Moreover, TBA is able to form adducts with sugars, nucleic acids and proteins, the absorbance maxima of which are very similar to that of TBA-MDA complex. Therefore, TBA test measures “TBA-reactive substances” instead of MDA and this is probably the reason for the inconsistent results obtained in different ischemia-reperfusion literature [23].

Ischemia-reperfusion has been shown to be associated with increasing oxidative stress in the course of surgery, which might be involved at the onset of atrial fibrillation, the most common arrhythmia after cardiac surgery. There are some hypotheses that supplementation of antioxidants like Alfa-Tocoferol might lower the incidence of atrial fibrillation after CABG surgery.

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Conflicts of Interest

Authors have declared that no conflicts of interest exist.

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