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RESEARCH ARTICLE

PROTEIN OXIDATION AND ANTIOXIDANT PARAMETERS IN LOW RISK AND HIGH RISK PATIENTS UNDERGOING OFF PUMP CORONARY ARTERY BYPASS GRAFT – A COMPARATIVE STUDY

[1]Sindhu, [2]Harish, [1]Jyothi MP D'souza, [3]Neevan D'souza and [1*]Vinitha Ramanath Pai

[1]Department of Biochemistry, Yenepoya Medical College, Yenepoya University, Mangalore, India
[2]Department of Cardiothoracic and Vascular Surgery, Yenepoya Specialty hospital, Mangalore, India
[3]Department of Community Medicine, Yenepoya Medical College, Yenepoya University, Mangalore, India

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ABSTRACT

Background: Coronary artery disease (CAD) patients scheduled for coronary artery bypass grafting (OPCABG) would represent the ideal situation to evaluate the potential injury induced by oxidative stress during ischemia and subsequent reperfusion. This study aims to compare markers of protein oxidation and antioxidants in serum of low risk and high risk patients, before and after undergoing OPCABG.

Methods: CAD patients scheduled for OPCABG - Group I: low risk patients (n=50) with ejection fraction > 40%, and group II : high risk patients (n=50) ejection fraction < 40% were included in the study. The mean values of the pre-operative and 24 hours post-operative levels of serum protein oxidation markers (advanced oxidative protein products, protein carbonyl) and antidoxidant parameters (total thiols, total antioxidant activity, total protein and albumin) were compared using ANOVA.

Results: In both groups, the post-operative levels of protein oxidation markers were significantly increased (pre-operative vs post-operative $p<0.01$), while the post-operative levels of the antioxidants parameters were significantly decreased ($p<0.01$) respectively. In each study group the post-operative levels of the protein oxidation markers were significantly higher than the pre-operative levels (low risk vs high risk group, pre-operative $p<0.01$, post operative $p<0.01$), with the antioxidants levels being significantly lower in the high risk group than in low risk group (low risk vs high risk group, pre-operative $p<0.01$, post operative $p<0.01$).

Conclusion: In patients undergoing OPCABG, the post-operative alterations in oxidative stress and antioxidants is suggestive of a need for similar vigilant clinical observation in both low and high risk groups to prevent post-operative complications.

INTRODUCTION

A higher incidence of coronary artery disease (CAD) in the developing world has increased the demand for coronary revascularization. This has generated an interest in off pump coronary artery bypass graft (OPCABG) which is one of the treatment modalities for CAD. OPCABG would represent the ideal situation to evaluate the potential injury induced by oxidative stress during ischemia and subsequent reperfusion, since, in such a clinical situation the time of onset and the duration of ischemia are known, as is the time and conditions of reperfusion. Moreover, all these conditions are under the control of the operating surgeon (Chambers, 2007). Oxidative stress leads to the damage of macromolecules such as lipids, proteins and DNA. Oxidation of proteins results in products such as protein carbonyl, advanced oxidative protein products (AOPP), etc., which are considered to be markers of oxidative stress (Bagatini *et al*, 2011; Skvarilova, 2005). The duration of post-operative alterations in oxidative stress after coronary artery bypass graft (CABG) is debatable.

In addition, intracellular antioxidant activity is reported to be a sensitive indicator of the overall health of the cell and its ability to resist toxic challenge. Hence, the aim of the study is to estimate and compare the pre-operative and post-operative levels of protein oxidation parameters (protein carbonyl and AOPP) and antioxidant parameters (thiols, total protein, albumin and total antioxidant activity) in both, low risk patients and high risk patients, undergoing OPCABG. The result may be suggestive of extent of necessary post operative clinical observation of both, the low and high risk groups, to prevent developments of complications.

Corresponding author: Vinitha Ramanath Pai
Department of Biochemistry, Yenepoya Medical College, Yenepoya University, Mangalore, India

Table 1. Comparison of pre- and post-operative levels of the markers of protein oxidation in serum of low risk and high risk patients of OPCABG (Mean ± SD)

	Study Groups					
	Low Risk Group of OPCABG (n=50)			High Risk Group of OPCABG (n=50)		
Parameters	Preoperative	Postoperative	p value	Preoperative	Postoperative	p value
AOPP (mmols/L)	132.6 ± 18.4	153.1 ± 29.2	*<0.01*	256.3 ± 48.8	308.1 ± 105.5	*<0.01*
Protein carbonyl (nmol/mg)	2.5 ± 0.6	2.9 ± 0.6	*<0.01*	4.1 ± 1.0	4.8 ± 1.2	*<0.01*

Table 2. Comparison of serum levels of the antioxidant parameters in the pre- and post-operative conditions of the study groups (Mean ± SD)

	Study groups					
	Low Risk Group OPCABG (n=50)			High Risk Group OPCABG (n=50)		
Parameters	Preoperative	Postoperative	p value	Preoperative	Postoperative	p value
Total thiols (mmols/L)	0.28 ± 0.06	0.24 ± 0.04	<0.01	0.2 ± 0.03	0.17 ± 0.04	<0.01
TAC (mmols/L)	1.8 ± 0.4	1.6 ± 0.4	<0.01	1.5 ± 0.3	1.3 ± 0.2	<0.01
Total proteins (g/dl)	6 .0 ± 0.5	5.7 ± 0.5	<0.01	5.3 ± 0.3	5.0 ± 0.4	<0.01
Albumin (g/dl)	4.3 ± 0.3	4.1 ± 0.3	<0.01	3.3 ± 0.14	3.1 ± 0.3	<0.01

MATERIALS AND METHODS

Study design

The study protocol was approved by the Institutional Ethics Committee. 100 patients with CAD referred to the cardiothoracic department of a tertiary care hospital from August 2012 till January 2013 was included in the study. Selected patients were aged between 40 - 75 years, and undergoing OPCABG, out of which 50 were low risk and 50 were high risk. Diabetics with no major complications were included in the study. A written informed consent was obtained from all patients the day before the surgery. The patients undergoing OPCABG were subjected to coronary angiography and echocardiography. The left ventricular function was assessed by echocardiography. The patients were divided into two groups - Group I and Group II.

Group I included low risk CAD patients (n=50) undergoing OPCABG with either one of the following criteria - good left ventricular function, i.e. ejection fraction > 40%, no major risk factors for surgery, having stable angina and no history of previous infarct.

Group Π included high risk CAD patients (n=50) undergoing OPCABG in the study centre. Patients identified with low ejection fraction (< 40%) were included in this group.

Exclusion criteria

Patients with valvular heart disease, ventricular aneurysm, heart failure, lung disease, cirrhosis, renal disease were excluded from the study.

Biochemical measurements

Blood samples were drawn for biochemical analysis from the cubital vein, 12 hour before operation and at 24 hours postoperatively. A part of the blood collected for routine diagnostic purpose was used in the study. The blood was transferred to EDTA bottles, shaken well and then centrifuged at 3000 rpm for 5 min, and the plasma was separated. The plasma thus collected was stored at -20°C until further analysis. Total thiols were analysed by GL Ellman's procedure (Ellman, 1959), AOPP by modified Witko's method (Witko, 1992), total antioxidant activity by spectrophotometric method of Koracevic *et al*.(2001), protein carbonyl by Levine's method as modified by Chakroborthy *et al* (2000), total protein by Biuret method (Varley and Gowenlock. 1953) and albumin by bromocresol green method (Webster,1974).

Statistical analysis

Data was analysed with the statistical package for social science version 11 (SPSS-11). The parameters were expressed as mean ± SD values. The significance of difference in the mean between groups and their comparison was calculated using Student's t-test and ANOVA. P value ≤ 0.05 was considered to be statistically significant.

RESULTS

The mean age of the patients in group I (low risk patients) was 53.06 ± 7.7 years and group II (high risk patients) was 58.1 ± 6.7 years. In the present study post-operative levels of protein oxidation markers, protein carbonyl and AOPP, were significantly increased ($p<0.01$) compared to pre-operative levels in both the low risk and high risk patients undergoing OPCABG as shown in table 1.

In addition the results also show that the post-operative levels of antioxidant parameters, i.e., total thiols, total antioxidant activity, total proteins, and albumin, were found to be decreased significantly ($p<0.01$) in both the study groups i.e., low risk OPCABG and high risk OPCABG patients as shown in table 2.

Comparison of the pre-operative levels of protein oxidation markers, protein carbonyl and AOPP, between the low risk and high risk patients undergoing OPCABG, showed that the levels were significantly higher ($p<0.01$) in the high risk group as shown in table 3.

The post-operative levels of protein oxidation markers, protein carbonyl and AOPP, in serum were also increased significantly ($p<0.01$) in the high risk compared to the low risk study group, as shown in table 4.

Table 3. Comparison of pre-operative levels of the markers of protein oxidation in serum of the study groups (Mean ± SD)

Parameters	Study Groups		
	Low risk OPCABG (n=50)	High risk OPCABG (n=50)	p value
AOPP (mmols/L)	132.6 ± 18.4	256.3 ± 48.8	<0.01
Protein carbonyl (nmol/mg)	2.5 ± 0.6	4.1 ± 1.0	<0.01

Table 4. Comparison of the post-operative serum levels of protein oxidation markers in the study groups (Mean ± SD)

Parameters	Study groups		
	Low risk OPCABG (n=50)	High risk OPCABG (n=50)	pvalue
AOPP (mmols/L)	153.1 ± 29.2	308.1 ± 105.5	<0.01
Protein carbonyl (nmol/mg)	2.9 ± 0.6	4.8 ± 1.2	<0.01

The pre-operative serum levels of the antioxidant parameters - total thiols, total antioxidant activity, total proteins, and albumin were significantly decreased ($p<0.01$) in the high risk group compared to the low risk group as shown in table 5.

Table 5. Comparison of the pre-operative serum levels of the antioxidant parameters in the study groups (Mean ± SD)

Parameters	Study groups		
	low risk OPCABG (n=50)	high risk OPCABG (n=50)	p value
Total thiols (mmols/L)	0.28 ± 0.06	0.2 ± 0.03	<0.01
TAC (mmols/L)	1.8 ± 0.4	1.5 ± 0.3	<0.01
Total proteins (g/dl)	6.0 ± 0.5	5.3 ± 0.3	<0.01
Albumin (g/dl)	4.3 ± 0.3	3.3 ± 0.14	<0.01

Comparison of the post-operative serum levels of antioxidant parameters between the study groups showed them to be significantly decreased ($p<0.01$) in the high risk group than the low risk group as shown in table 6.

Table 6. Comparison of the post-operative levels of antioxidant markers in serum of the OPCABG study groups

Parameters	Study groups		
	low risk OPCABG (n=50)	high risk OPCABG (n=50)	p value
Total thiols (mmols/L)	0.24 ± 0.04	0.17 ± 0.04	<0.01
TAC (mmols/L)	1.6 ± 0.4	1.3 ± 0.2	<0.01
Total proteins (g/dl)	5.7 ± 0.5	5.0 ± 0.4	<0.01
Albumin (g/dl)	4.1 ± 0.3	3.1 ± 0.3	<0.01

DISCUSSION

OPCABG is a widely used surgical treatment of CAD (Chandrasena *et al*, 2009). The major concern during this procedure is the preservation of the myocardial function. Free radicals are produced as a result of myocardial ischemia which is seen during cardiac surgery (Castillo Caparrós, 2005). Although protein and lipids and DNA are known to undergo oxidation, protein oxidation does not have the features of a chain reaction as seen in lipid peroxidation (Seema *et al*, 2010). Free radicals modify amino acid side chains of proteins such as arginine, lysine, threonine and proline residues to form protein carbonyls (Seema *et al*, 2010). The dityrosine-containing protein crosslinking products formed due to oxidation of amino acids are designated as AOPP (Witko-Sarsat *et al*., 1996). Therefore, the evaluation of protein carbonyl and AOPP as biomarkers of oxidative stress has some advantages in comparison with the measurement of other oxidation products. In addition, they are formed relatively early and are extremely stable. In the present study it was observed that protein carbonyl and AOPP levels were significantly increased in low risk patients of OPCABG, 24 hours postoperatively, compared to the pre-operative levels, supporting previous study of Gonenc *et al*. (2011), who also observed elevated protein carbonyl levels following OPCABG.

This finding supports the evidence that oxidative stress remains a constant underlying factor that will influence clinical outcome as long as meticulous myocardial protection is provided and the ischemic duration is kept as short as possible. The 24 hours post-operative levels of protein carbonyl and AOPP levels were also found to be significantly higher in the high risk patients compared to their pre-operative levels, supporting the speculation that high-risk patients are more likely to experience harm from the continued oxidative stress even after reperfusion. This could be because duration of the surgery is usually longer in the high risk group since the heart tissue is more compromised, leading to higher levels of free radicals being generated. Patients with already extensive disease of the heart would therefore, probably be more vulnerable to further damage by free radicals. The findings of this study also indicate that, even the low risk groups are as susceptible to the oxidative stress as the high risk group. Although there is reperfusion of ischemic myocardium by OPCABG, depletion of antioxidants is concomitant with the continued generation of free radicals as evidenced by significant decrease in the levels of the antioxidant parameters - total thiols, albumin, total proteins and total antioxidant activity 24 hours postoperatively compared to pre-operative levels in the low risk patients undergoing OPCABG.

The major part of thiols in plasma is derived from proteins, especially albumin. Significant low levels of total thiols after reperfusion may indicate increased consumption of these thiol groups to neutralize the increased levels of reactive oxygen species as suggested by Mehmet *et al*., (2013). It may not be wrong to speculate that oxidation of albumin may have lowered not only the plasma albumin levels but also the thiol levels in the patients undergoing OPCABG. On the other hand, the significant decrease of total antioxidant activity in the post-operative period compared to the pre-operative levels, in the low risk patients, is in agreement with the previous study of Danova *et al*., (2005). We also observed substantial depletion in the antioxidant parameters, i.e., total thiols, albumin, total proteins and total antioxidant activity 24 hours post-operative levels compared to pre-operative levels in high risk patients undergoing OPCABG. In conclusion, increased levels of

protein carbonyl and AOPP accompanied by decreased total thiols, albumin, total proteins and total antioxidant activity in serum, 24 hours post-operatively compared to the pre-operative day in both low risk and high subjects undergoing OPCABG suggests reciprocal alterations in oxidative stress and antioxidant activity which necessitates vigilant clinical observation of the same extent in both the low risk and high risk group after OPCABG to prevent post-operative complications.

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