

Original Article

Oxidative stress and homocysteine metabolism following coronary artery grafting by on pump and off pump CABG techniques

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Abstract

Objectives: To compare the effect of on-pump and off-pump CABG on the induction of the oxidative stress and the metabolism of homocysteine, which is involved in the synthesis of glutathione.

Methods: This retrospective study was performed in Shahid Madani Heart Hospital in Tabriz, Iran in 2004 using a questionnaire. Plasma homocysteine, folate total antioxidant capacity (TAC) and malondialdehyde (MDA) were determined on blood samples obtained from 40 patients undergoing CABG, preoperatively and at 0,12,48,120 hours and 6 months after surgery. The patients were divided into two matched groups, one off-pump and the other on-pump CABG.

Results: A marked reduction of homocysteine, folate and significant elevation of MDA were noticed at 0, 12, 48 hours after operation in the both groups ($P<0.05$). A negative and marked correlation between homocysteine and TAC but a positive and significant between homocysteine and MDA were observed ($P<0.05$ in the both groups).

Conclusion: In CABG operation because of oxidative stress and consumption of GSH immediate reduction in the plasma levels of homocysteine occurs in the both techniques. However using off pump CABG induction of oxidative stress and changes in plasma levels of homocysteine are not as high as on- pump CABG. (Rawal Med J 2007;32:3-7)

Key Words: CABG, CPB, homocysteine, folate, oxidative stress.

INTRODUCTION

Coronary artery bypass grafting (CABG) has been recognized as a major cause of systemic inflammatory reaction that is believed to result in global ischemia of whole body; increased postoperative morbidity and prolonged hospital stay.¹ Several adverse postoperative outcomes such as renal, pulmonary and neurological complications, bleeding and multiple organ dysfunctions have been reported.² Experimental and clinical studies have shown that free radicals are generated in the myocardium during coronary bypass surgery^{3,4} and much information is now available about the role played by oxygen free radicals in the etiology of myocardial dysfunction after ischemia and reperfusion.^{5,6} Hyperhomocysteinemia is widely regarded as a risk factor for arterial⁷ as well as venous thrombosis.⁸ Homocysteine is a product resulting from the metabolism of amino acid methionine.

The Trans-sulfuration pathway, whose starting substrate is homocysteine, converts methionine to cysteine, which is in turn converted to glutathione.⁹ Some investigators have shown that homocysteine levels tend to increase during the first weeks or months after cardiac surgery procedures,^{10,11} while may be reduced in general surgical patients early postoperatively.¹² Recently, off-pump CABG has gained widespread use as alternative techniques to conventional on-pump CABG. The avoidance of cardiopulmonary bypass (CPB) and myocardial ischemia-reperfusion has been proposed to significantly reduce the postoperative systemic complications which negatively affect the preoperative course after surgical myocardial revascularization.^{13,14} The present study was designed to investigate and compare oxidative stress, lipid peroxidation and homocysteine metabolism following coronary artery grafting by on- pump and off- pump techniques.

METHODS

In 2004 forty patients, 33 men and 7 women with two vessel coronary artery disease undergoing elective CABG were included. We compared 20 patients (16 males and 4 females) who underwent off-pump surgery with 20 patients (16 males and 4 females) who underwent on pump surgery in a consecutive enrolment design. Patients with diabetes, diseases of the circumflex and left main stem, valvular diseases, ventricular aneurysm, heart failure, and poor left ventricular function were excluded from the study. Since altered renal function, known to effect the homocysteine concentration, postoperative acute renal failure (creatinie > 2.0 mg/dl at any time after the operation) patients were excluded.

Blood samples for plasma homocysteine, folate, total antioxidant capacity (TAC), malonedialdehyde (MDA), total protein and creatinine measurements were obtained from all patients at hospital admission, at the end of surgery and then at 12 , 48 and 120 hours postoperatively and also at 6 months follow up. Plasma concentration of homocysteine was measured by high performance liquid chromatography.¹⁵ Total antioxidant capacity in plasma samples was assessed using the randox total antioxidant status kit and the level of MDA was measured by a spectrophotometrical method.¹⁶ Radioimmuno–assay method (Bio-source kit) was used for folate evaluation and plasma total protein, creatinine and glucose were measured with standard methods in the cobas mira autoanalyzer.

SPSS 11 was used to perform statistical analysis. Paired student's t-test was employed to determine the significance of differences between the measured parameters. Comparisons between different groups were made using ANOVA and correlations were evaluated by linear regression. Results are expressed as mean \pm SD and statistical significance was set at $P < 0.05$.

RESULTS

The clinical characteristics and operative data are shown in table 1. There was no hospital mortality, no pulmonary insufficiency, neurologic accidents or any myocardial infarction in the two study groups. All the patients were extubated between 4 and 14 hours after their arrival in the intensive care unit. The mean time of mechanical ventilatory support was 10.8 ± 1.5 hour in the on-pump group, whereas it was only 6.7 ± 0.5 hours in the off-pump group ($P < 0.05$). The postoperative blood loss through the chest drains was 642 ± 49 ml in the on pump group and 462 ± 45 ml in the off pump group ($P < 0.03$). Blood transfusion was performed when hemoglobin levels fell below 10g/dl and this was required in 6 cases in on pump group and only in one case in the off pump group.

Postoperative fever occurred in 11 patients in the on pump group and only in 4 patients of the off pump group (P<0.05).

Table 1. Clinical characteristics and preoperative data

Variable	On pump	Off pump	P value
Age (years)	56 ± 10.8	54 ± 9.4	NS
Male to female (ratio)	17:3	16:4	NS
Hypertension (no-of patients)	4	6	NS
No. of grafts / patients	1.5 ± 0.2	1.9 ± 0.2	NS
Ischemic time (min)	37.3 ± 35	38.5 ± 46	NS
CPB time (min)	71.6 ± 6.4	0	<0.05
CPB: Cardiopulmonary bypass, NS: No Significant, Min: Minute			

The mean of preoperative total antioxidant capacity were similar for both groups (table 2). A significant decrement in the mean levels of total antioxidant capacity in the on pump and off pump groups was observed at end of operation, 12 h and 48 h after surgery (P<0.05). The serum total antioxidant reached the minimum level 12 h postoperatively and the reduction in off pump group was markedly higher than that of on pump group (P<0.001). The mean MDA concentrations in the both groups was significantly high at the end of operation 12h and 48 h samples and maximum elevation was noticed at 12 h samples (P<0.05). The elevation in on-pump group was more higher than that of off- pump group (P<0.0001).

Table 2. Total antioxidant capacity and MDA levels in the both groups of the patients

Time of Sampling :	Total antioxidant capacity (mmol /L)		MDA (mmol/L)	
	Off pump	On pump	Off pump	On pump
Admission	1.55±0.19	1.49±0.18	2.41±0.40	2.22±0.32
End of operation	1.22±0.15*	0.99±0.18*	3.60±0.22*	6.38±1.35*
12 hours	1.15±0.65* ⁺⁺	0.81±0.055*	4.5±0.31* ⁺⁺	6.8±1.05* ⁺⁺
48 hours	1.43±0.17 ⁺⁺	1.26±0.085* ⁺⁺	4.3±0.63*	3.89±0.88* ⁺⁺
120 hours	1.45±0.15	1.47±19	2.55±0.32 ⁺⁺	2.25±0.45 ⁺⁺
6 months	1.58±0.17	1.53±0.13	2.38±0.24	2.15±0.18
P<0.001 VS. Admission		++ P<0.001 VS. Previous time point		MDA: Malone Dialdehyde

The mean ± SD values of plasma homocysteine and folate in the both groups at admission in the hospital, soon after end of surgery, 12, 48, 120h and 6 months after the surgery are shown in table 3. A meaningful decrease in both homocysteine and folate was noticed during and several hours after the surgery in the off-pump and on-pump groups (P<0.0001 in the both cases). The plasma homocysteine levels reached the minimum level 12 hours after CABG and then returned near to the baseline levels at 48 hours samples. At 12 hours the reduction in the plasma levels of homosysteine in on pump group was significantly higher than that of off pump group (P<0.001). The elevation of the homocysteine levels at 48 in the on pump group was markedly faster and higher than that of off pump group (P<0.001). No significant differences between homocysteine levels of 120 hours, 6

months follow up, and baseline levels were noticed ($P>0.05$). The mean level of folate reached the lowest values 48 hours postoperatively and was still markedly reduced on postoperative day 5 in the both groups. Significant differences were noticed between the reduction in the folate levels using the two different techniques of operation ($P<0.05$).

Table 3. Plasma concentrations of homocysteine and folate in the two groups of patients

Time of Sampling	Homocysteine (Micromole/L)		Folate (mg/ml)	
	groups		groups	
	Off pump	On pump	Off pump	On pump
Admission	16.5±2.4	15.9±1.8	5.1±1.6	4.9±1.72
End of operation	11.8±17*	8.2±1.2*	4.9±1.7	4.8±1.54
12 hours	7.5±0.85* ⁺⁺	5.1±0.56* ⁺⁺	3.8±2.1* ⁺⁺	3.35±1.6* ⁺⁺
48 hours	12±1.1* ⁺⁺	14.5±1.5* ⁺⁺	3.2±1.68* ⁺⁺	2.8±1.42* ⁺⁺
120 hours	15±2.0* ⁺⁺	16.1±2.2	4.5±2.2* ⁺⁺	4.0±1.68* ⁺⁺
6 months	16.1±2.8	16.4±1.65	5.5±1.8* ⁺⁺	5.35±1.2* ⁺⁺

* $P<0.001$ VS. Admission ++ $P<0.001$ VS. Previous time point

The correlation between the levels of homocysteine and those of other measured parameters was determined in 12 hours postoperative samples with minimum and maximum changes in the concentrations of the parameters. A positive and significant correlation was noticed between homocysteine and MDA (table 4). A negatives and marked correlation was present between homocysteine and total antioxidant capacity. No correlation was observed among homocysteine and folate. The correlations between the parameters in other samples were not significant ($P>0.05$).

DISCUSSION

CABG with use of CPB causes ischemia and oxidative stress of the whole body.¹⁷ A more radical and effective way of counteracting the effects of the inflammatory reaction and oxidative stress may be the omission of CPB itself. Markedly greater decrease in the level of total antioxidant capacity and significantly higher increase in the concentration of MDA in the on pump CABG procedure may be partially due to reperfusion of heart following global cardiac arrest, oxidative stress and inflammation.¹⁸

Table 4. Correlation analysis between plasma levels homocysteine and other measured parameters in 12 hours postoperative samples in on- pump and off- pump groups.

Parameters	On pump group		Off pump group	
	r	P value	r	P value
Homocysteine – MDA	0.815	<0.05	0.640	<0.05
Homocysteine- total Antioxidant capacity	-0.640	<0.01	-0.621	<0.01
Homocysteine – folate	0.356	NS	0.220	NS

NS: No Significant r: regression

Oxidative stress following CABG may lead to an increased consumption of GSH and of homocysteine. One of the main determinants of the rate of GSH synthesis is the availability of cysteine which comes from homocysteine through the transsulfuration pathway. Therefore, ischemia–reperfusion related oxidative stress may induce a reduction in plasma levels of both cysteine and homocysteine. Because of high oxidative stress in on-pump group, the reduction in the levels of the parameters was higher than off-pump group ($P < 0.001$). It seems that high oxidative stress in on pump technique causes high reduction of homocysteine levels in this group of patients. Increased levels of homocysteine 6 days to 6 weeks after CABG has been reported¹⁰ and postulated that since homocysteine is a known risk factor for atherosclerosis, these changes may be importance to the pathophysiology of vein graft disease. Other investigators found a similar increase of the homocysteine levels 3-6 months after heart transplantation, and speculated that hyperhomocysteinemia following surgery could increase the development of cardiac allograft vasculopathy.¹¹ The reduction of homocysteine concentrations in the early postoperative period after general surgical procedures has also been reported¹² and homocysteine is considered a negative acute phase reactant. In the present study, a marked postoperative reduction of plasma homocysteine levels was noticed in patients undergoing on-pump and off-pump techniques of CABG surgery. The percentage of the reduction in on-pump group was meaningfully higher than the off-pump group. We found that homocysteine levels were not different from baseline at 6 months follow up in the both groups. In the case of on pump technique, similar results have been reported by others.¹⁹

Our data confirm the observation that the reduced form of GSH acts as first line of defense against oxidative stress. The levels of folate reached the minimum value 48 hours after operation and were still meaningfully reduced in 120 h samples, but at 6 month follow up comparing with baseline levels no differences was observed. In conclusion, our results indicate that the on and off pump techniques of operation induce oxidative stress but the off-pump bypass graft procedure causes significantly less oxidative stress. The data may define one of the beneficial effects of graft operation by off pump technique and usefulness of antioxidant supplementation before CABG operation.

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