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Araştırma / *Research Article*

Comparison of Myocardial Metabolism and Apoptosis in Patients Undergoing CABG Operation Performed Either with Fibrillation or Cardioplegia Method §

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Fibrilasyon veya Kardiyopleji Metoduyla KABG Operasyonu Yapılan Hastalarda Miyokardiyal Metabolizma ve Apopitozisin Karşılaştırılması

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ABSTRACT

Objective: *Optimal myocardial protection during CABG operations is still debatable. In this study we compared hemodynamic data, myocardial oxygen extraction, lactate production and presence of apoptosis in transmural biopsy specimens in operations performed either with intermittent aortic cross-clamping with fibrillation (IAC) or with cardioplegic cardiac arrest (CCA) method.*
Methods: *Following Ethics Committee approval, consecutive patients with normal ventricular function and without any comorbid disease other than regulated hypertension and/or type-2 DM were included in the study (IAC group n=13, CCA group n=8). Hemodynamic data, serial CK-MB values and ECG changes at intermittent time points were recorded till postoperative 24 hours. Myocardial oxygen extraction and lactate production were calculated from the blood samples withdrawn from the aortic root and the coronary sinus cannula before total CPB, during cross-clamping and following removal of cross-clamp. Transmural biopsy specimens obtained before total CPB and cessation of CPB were analyzed for apoptosis.*
Results: *Demographic and intraoperative hemodynamic variables were similar. Pre ischemic and post ischemic myocardial oxygen extraction and lactate production values were comparable in both groups. Seven patients in IAC and one patient in CCA group needed low dose inotropic support during early postoperative period. Any evidence of apopitosis was not found in both groups.*
Conclusion: *This study shows that for low risk CABG procedures, IAC and CCA methods were not superior to each other in protecting the myocardium from ischemic injury with respect to myocardial oxygen extraction, lactate production and presence of apoptosis.*

Keywords: *cardiac surgery, myocardial protection, oxygen extraction, lactate production*

ÖZ

Amaç: *Koroner arter baypass (KABG) operasyonlarında, özellikle değişik teknikler kullanıldığında optimal miyokardiyal koruma halen tartışmalıdır. Bu prospektif çalışmada, fibrilasyonda aralıklı aortik kros-klempleme (IAC) tekniği veya kardiyoplejik kardiyak arest (CCA) tekniği ile yapılan elektif KABG vakalarını, miyokardiyal metabolizma ve transmural biyopside apopitoz varlığı açısından karşılaştırmayı amaçladık.*
Yöntem: *Etik komite onayı ile ardarda gelen elektif KABG operasyonu geçirecek normal ventrikül fonksiyonlu, regüle hipertansiyon ve/veya tip II DM dışında başka komorbid hastalığı olmayan IAC grubunda 13 hasta, CCA grubunda 8 hasta ile çalışma yapıldı. Hemodinamik veriler, aralıklı CK-MB değerleri ve ECG değişiklikleri postoperatif 24 saate kadar kaydedildi. Miyokardiyal oksijen ekstraksiyonu ve laktat üretimi, aort kökü ve koroner sinüse yerleştirilen retrograd kanülden; kardiyopulmoner baypas (CPB) dan hemen önce, kross-klemp sırasında ve kross-klemp kaldırılmasını takiben 5-10dk. içindeki dönemlerde alınan kan örneklerinden hesaplandı. Sol ventrikül ön duvarından CPB öncesi ve sonrası alınan biopsiler apopitoz açısından incelendi.*
Bulgular: *Demografik ve intraoperatif hemodinamik veriler gruplar arası benzerdi. Her iki grupta da iskemi öncesi ve iskemi sonrası miyokardiyal oksijen ekstraksiyonu ve laktat üretimi benzerdi. IAC grubunda yedi hastada ve CCA grubunda bir hastada erken postoperatif dönemde düşük doz inotrop ihtiyacı oldu. Her iki grupta apopitozu gösterir bir kanıt bulunamadı.*
Sonuç: *Bu çalışma bize düşük risk grubundaki KABG operasyonlarında kullanılan IAC ve CCA metodunun miyordiyal koruma ve apopitoz varlığı açısından birbirlerine üstünlükleri olmadığını gösterdi.*

Anahtar kelimeler: *kardiyak cerrahi, miyokardiyal koruma, oksijen ekstraksiyonu, laktat üretimi*

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INTRODUCTION

Optimal myocardial protection methods during coronary artery bypass grafting (CABG) operations are still debatable. For routine CABG, performed on cardiopulmonary bypass (CPB) cold blood cardioplegia; ie. cardioplegic cardiac arrest (CCA) method is probably the worldwide standard used by the majority of surgeons. Although considered as an old method, a minority of surgeons use intermittent ischemia and induced ventricular fibrillation; intermittent aortic cross-clamping with fibrillation (IAC) method [1,2]. We hypothesized that CCA method is not superior to IAC in terms of myocardial protection during CPB.

In our hospital CCA method is routinely used while IAC method was brought into practice by a transferred surgeon. We performed this study for comparing the cardioprotective effect of these two techniques for low-risk CABG operations done consecutively. We recorded the hemodynamic data for detecting symptoms of ischemia. On the other hand during CPB period in these two operation techniques, we compared myocardial metabolism markers such by calculating myocardial oxygen extraction and lactate production [3]. Cardiomyocyte apoptosis occurs in ischemic myocardial tissue injury and has been shown to occur in human acute myocardial infarction [4]. Therefore we examined whether an apoptotic type of cell death occurs in human left ventricle transmural biopsy specimens in both groups to compare the cardioprotective effect of these two methods. There is less data in humans related to the anaerobic; lactate production and aerobic metabolism; oxygen extraction of myocardium and apoptosis, especially during IAC method while on CPB. This study is unique with this respect as it compares a very rarely used IAC method with a worldwide used CCA cardioprotection method.

MATERIAL and METHODS

Study was started after our hospital ethics committee gave permission to the study design. American Society of Anesthesiologists (ASA) physical status II patients without any co-existing disease other than regulated hypertension or type II diabetes mellitus (DM), who have or had two or three vessel disease, and scheduled for their first CABG operation and were included in the study. ASA ≥ II patients with left ventricular ejection fraction (EF) lower than 35% without any other co-existing disease, and needed emergent operations were excluded from the study.

After informed consent was obtained, consecutive patients who met the inclusion criteria were included in the study. Patients in the CCA (n=8) group had all of their operations performed by the same surgeon using CCA method which is a routine practice in our cardiovascular surgery clinic;. Another group of patients were operated all by the transferred surgeon using the IAC method (n= 13).

Patients' radial arteries were cannulated for invasive blood pressure monitorization. Then anesthesia induction was done via intravenous route with lidocaine 1 mg/kg, midazolam 0.1 mg kg^{-1}, fentanyl 10-15 μgr^{-1} kg^{-1} and rocuronium 0.5-0.7 mg^{-1} kg^{-1}. Following endotracheal intubation, the right internal jugular vein was cannulated for central venous pressure monitorization. Anesthesia was maintained with intermittent fentanyl-midazolam bolus injections and sevoflurane was used at a dose of 1 MAC during off-pump period. Rocuronium was used as muscle relaxant when needed.

Patients in both groups were cooled to 32°C during CPB. Following partial CPB, a retrograde cannula was inserted into coronary sinus in both groups. Baseline blood samples were withdrawn from coronary sinus retrograde cannula and aortic root cannula in both groups for calculating myocardial oxygen extraction and lactate production.

In the CCA group St. Thomas' Hospital cardioplegic solution was infused using a manual pump into the aortic root with a pressure of 80 mmHg at 6-9°C. In retrograde delivery the pressure was 30-40 mmHg.

Initially a total amount 10 ml/kg of solution was infused equally half and half via antegrade and retrograde cannulas. Then every 20-30 min. approximately 400 ml of cold blood cardioplegia solution at +4°C was infused via retrograde cannula with 200 ml/min flow and finally hot shot of 400 ml of warm (37°C) blood cardioplegia was perfused just before removal of aortic cross-clamp.

In the IAC group, pacing wires were localized in the ventricle and attached to a fibrilator device. Distal anastomosis was done while the heart was fibrillating. Then aortic cross-clamp was removed, heart was defibrillated and the proximal anastomosis was done while the heart is being reperfused. The same procedure was repeated for every graft anastomosis.

Demographic data, co-existing disease(s), echocardiography and angiographic findings were recorded. Intraoperative cross-clamping and cardiopulmonary bypass (CPB) periods, positive inotrophic agent administration or intraaortic balloon pump usage were recorded. Intraoperative heart rate (HR), ECG, mean arterial pressure (MAP), central venous pressure (CVP), and urine output were also assessed.

Phosphocreatine kinease MB fraction (CKMB) were measured preoperatively and 6 and 24 hours postoperatively. Several blood samples were obtained during CPB. Myocardial arterial blood samples were withdrawn through the aortic root cannula and retrograde coronary sinus cannula was used for venous blood sampling. 1) during partial CPB, before placement of cross-clamp in both groups. 2) following placement of, cross-clamp before fibrillation in IAC and after cardioplegia infusion in CCA group. 3) during rewarming for 5 minutes following removal of cross-clamp. These blood samples were analyzed to calculate myocardial oxygen extraction and lactate production. We define myocardial oxygen extraction as oxygen content of the arterial blood (aortic root) minus oxygen content of the coronary venous effluent (coronary sinus blood obtained from retrograde cannula). Myocardial lactate production was defıned as the lactate concentration of the coronary venous effluent minus the lactate concentration of the aortic root blood.

Postoperative positive inotrophic agent administration, intraaortic balloon pump usage, arrhythmias like atrial fibrillation (AF) in the ICU were all recorded. ICU and hospital stay and any other complications were also recorded.

Biopsy specimens were obtained from the anterior wall of the left ventricle approximately 5cm above the apex, by Tru-Cut biopsy needle. Specimens were obtained before initiating CPB and after the patients were rewarmed to a rectal temperature of 35°C but before cessation of CPB. Specimens were put into formaldehyde solution for 3 days than stored in 70% alcohol at 4°C and examined by an experienced pathologist for the presence of apoptosis.

Histopathology and Immunohistochemistry: The cardiac biopsy tissue samples were blocked and processed on a 2- hour-schedule and embedded in paraffin. The five-micron sections were cut and mounted onto charged slides. From each block, one hemotoxylin and eosin stained slide was prepared. Immunohistochemical staining for caspase-3 (Biocare Medical, Walnut Creek, California) was performed as follows: five-micron sections were flushed with water using a standard protocol. Following a through rinsing procedure, antigen was retrieved by incubation in a steamer for thirty minutes using ReVeal buffer with a pH 6.0. After a ten-minute cool down period, the slides were rinsed and mounted onto holder and put onto Autostainer, TechMate Capillary Gap System. The primary antibody was used at a dilution of 1:200 and incubated at four degrees overnight. Secondary biotinlayted antibody (Signet) was incubated at room temperature for twenty minutes, followed by treatment with H_2O_2 blocking agent for ten minutes. Slides were incubated with streptavidin (Signet) for twenty minutes at room temperature, followed by DAB staining (Signet) at room temperature for one minute. Hematoxylin stain (Surgipath)

was used for counterstaining for one minute) The slides were then dehydrated, cleared, and coverslipped. Under light microscopy areas with intense myocyte population were examined. Punch biopsies of skin and high -grade breast cancer tissue were used as positive control apoptosis was determined by the positive staining apoptotic myocyte nuclei under light microscopy examined by two experienced pathologists.

Statistical analysis

Statistical analyses was performed using IBM SPSS Statistics 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). P<0.05 was considered as an indicator of significant difference in statistical decisions. Preoperative and postoperative parameters were compared between the groups and the results were also compared in the groups for each time point. Chi-square and independent samples- t tests were used to determine the statistical differences between two groups. Wilcoxon test was used to evaluate the differences in the same group during the time periods. Nonparametric tests were used in the presence of non-homogeneous data that results from limited number of patients. A p value less than 0.05 was considered statistically significant (p<0.05). All values reported as mean±SD.

RESULTS

Thirteen consecutive patients in the IAC group and eight patients in the CCA group who met the inclusion criteria were included in the study.

The two groups were similar as there was no statistical difference between them with respect to age, gender, EF, angiographic lesions and number of bypassed vessels (Table 1). Total cross-clamping and CPB times were similar between the groups (Table 1). Five patients in the IAC and 3 patients in the CCA group had a history of previous MI but none of the patients had EF below 35%.

Hemodynamic variables, heart rate, ECG, MAP, CVP, urine output within time periods like preinduction,

Table 1. Demographic data and intraoperative findings.

Variable	CCA (n=8)	IAC (n=13)	p Value
Age (year)	53.12 ±11.48	58±9.8	0.226
Gender (n)			
Male	8	12	
Female	0	1	
Co-existing disease (n)			
None	3	3	
DM	2	1	
HT	3	6	
DM + HT	0	3	
Preoperative EF (%)	57.87±5.3	54.07±6.1	0.162
The number of by-passed vessels			
One vessel (n)	3	1	
Two vessels (n)	3	9	
Three vessels (n)	2	3	
Cross-clamp time (min.)	31.62±13.2	24.69±7.1	0.132
Total CPB time (min.)	58±24.74	68±19.4	0.314

Values are expressed as mean±SD. CPB: cardiopulmonary bypass; DM: diabetes mellitus; EF: ejection fraction; HT: hypertension.

Table 2. Heart rate (beats/min) during selected time periods.

Heart Rate (beat/min)	CCA (n=8)	IAC (n=13)	p Value
Preinduction	85.75±13.6	86.23±18.29	0.949
Termination of CPB	91.5±16.01	96.6±14.5	0.461
Postoperative 6th hr	103 ±17.3	97.38 ±7.9	0.319
Postoperative 24th hr	92.6±10.3	100.3±12.3	0.156

Values are mean±SD. CCA: cardioplegic cardiac arrest; CPB: cardiopulmonary bypass; IAC: intermittent aortic cross-clamping with fibrillation. There was no significant difference between the groups.

Table 3. Mean arterial pressure (mmHg) during selected time periods.

MAP (mmHg)	CCA (n=8)	IAC (n=13)	p Value
Preinduction	107.87±18.5	106±10.5	0.759
During CPB	61.25±6.4	62.3±2.5	0.598
Termination of CPB	65.5±10.9	62.23±6.5	0.397
Postoperative 6th hr	90.25±16.1	92.15±15.4	0.790
Postoperative 24th hr	78.5±10.2	81.5±9.9	0.513

Values are mean±SD. CCA: cardioplegic cardiac arrest; CPB: cardiopulmonary bypass; IAC: intermittent aortic cross-clamping with fibrillation. There was no significant difference between the groups.

Table 4. Central venous pressure (mmHg) during selected time periods

CVP (mmHg)	CCA (n=8)	IAC (n=13)	p Value
Preinduction	5.5±3.7	7.4±1.7	0.123
Termination of CPB	4,1±3.2	5.6±2.6	0.254
Postoperative 6th hr	3.3±2.3	4±2.3	0.506
Postoperative 24th hr	3±1.6	2.4±1.5	0.396

Values are mean±SD. CCA: cardioplegic cardiac arrest; CPB: cardiopulmonary bypass; CVP: Central venous pressure; IAC: intermittent aortic cross-clamping with fibrillation. There was no significant difference between the groups.

Table 5. Urine output (ml/hr) during selected time periods.

Urine Output (ml/hr)	CCA (n=8)	IAC (n=13)	p Value
Preinduction	93.7±34.7	114.2±68.9	0.446
During CPB	206.2±147.4	317.3±206.5	0.202
Postoperative 24 hr	171.8±24.7	169.2±59.6	0.908

Values are mean±SD. CCA: cardioplegic cardiac arrest; CPB: cardiopulmonary bypass; IAC: intermittent aortic cross-clamping with fibrillation. There was no significant difference between the groups

Table 6. Creatine Kinase, MB Form (U/l) values during selected time periods.

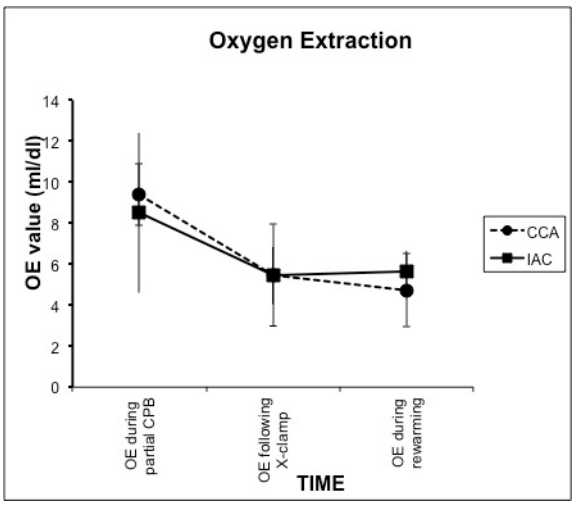
CK-MB (U/l)	CCA (n=8)	IAC (n=13)	p Value
Preinduction	17.2±4.4	13.4±4.2	0.063
Postoperative 6th hr	60.3±34.8	89.5±70	0.288
Postoperative 24th hr	41±23.9	71.7±61	0.193

Values are mean±SD. CCA: cardioplegic cardiac arrest; CK-MB: Creatine Kinase, MB Form; CPB: cardiopulmonary bypass; IAC: intermittent aortic cross-clamping with fibrillation. There was no significant difference between the groups.

during the CPB, following CPB, 6th hour and 24th hour in the ICU were similar between the groups (Tables 2-5).

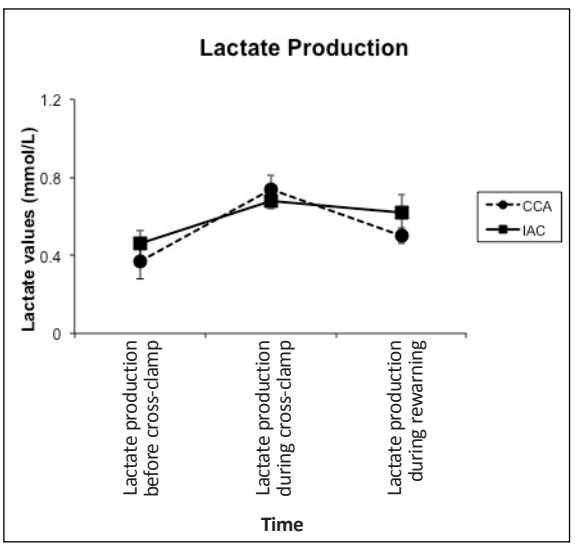
Parameters of myocardial metabolism during ischemia and reperfusion: in both groups were similar in terms of preischemic (before cross-clamping) and postischemic (after cross-clamping) myocardial oxygen extraction and lactate production (Figure 1, 2).

Creatine kinase (CKMB) values were normal during all time periods and showed no difference between both groups (Table 6). Seven patients out of thirteen needed low dose inotropic support (dopamine 5 mcgr/kg/min) during early postoperative period in the IAC group while only one patient needed dopamine in the CCA group. None of the patients required intra-aortic balloon pump (IABP) postoperatively. All of the patients in both groups discharged from the ICU on the postoperative day one except



OE: Oxygen Extraction ; CCA: cardioplegic cardiac arrest; CPB: cardiopulmonary bypass; IAC: intermittent aortic cross-clamping with fibrillation. There was no significant difference between the groups.

Figure 1. Oxygen Extraction (ml/dl) during selected time periods.



CCA: cardioplegic cardiac arrest; CPB: cardiopulmonary bypass; IAC: intermittent aortic cross-clamping with fibrillation. There was no significant difference between the groups.

Figure 2. Lactate production (mmol/L) during selected time periods.

two from the IAC group who were discharged on the second and third postoperative days. First patient received dopamine for 32 hours and the other patient for two days and had atrial fibrillation postoperatively.

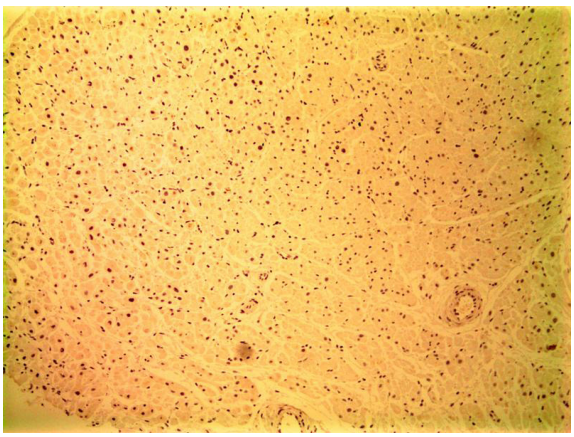


Figure 3. Immunohistopathologic examination of myocardial tissue biopsy. No apoptotic nuclei were detected.

Apoptosis was determined by immunohistochemical examining of the positive stained apoptotic myocytes under light microscopy by two experienced pathologists. In both groups none of the cardiac muscle nuclei showed staining under light microscopy (Figure 3).

DISCUSSION

Myocardial protection during CABG surgery remains to be evaulated especially for high risk patients. We want to point out the comparison between a relatively uncommon method; IAC technique and CCA method for CABG operations regarding their effectiveness in myocardial protection in consideration of clinical and some laboratory data.

In this study myocardial metabolic activity during CPB was assessed with estimation of myocardial oxygen extraction and lactate production during ischemia and reperfusion periods. Evaluation of apoptosis in myocardial transmural biopsy specimens allowed us to detect the myocardial necrosis, if any. Perioperative hemodynamics and CKMB values were detected to enlighten some symptoms of ischemia if occurred. Postoperative early outcome was also assessed. This study is unique because there is less data in humans describing the metabolism of myocardium and apoptosis during CPB.

In our study hemodynamic data were similar between the groups throughout the operation and postoperative 24th hour. Gerola et al. performed a prospective randomized controlled trial in 60 elective CABG patients to compare the efficiency of IAC versus CCA with hot shot (Buckberg method) cardioplegia with respect to hemodynamic parameters and enzyme levels. They could not find any difference between these groups and concluded that the two methods had similar effectiveness as for myocardial protection [5]. Alhan et al. conducted, a similar study with 40 low-risk patients and additionally studied ultrastructural changes in left ventricle biopsy specimens. They concluded that either technique was safe in low-risk CABG patients [6].

It is known that lactate production is a reflection of anaerobic metabolism while oxygen extraction shows the aerobic state of metabolism. There are articles about myocardial oxygen supply:demand index while resting and during heart disease [7]. Myocardial glucose and lactate metabolism during rest and atrial pacing in humans were clarified with some aspects [8]. The effect of some agents like nitric oxide on oxygen demand-supply balance in right ventricular disease were also assessed [9]. However there is less data in humans describing the anaerobic and aerobic metabolism of myocardium during CPB. This study is unique as it compares two different myocardial protection methods with this respect. We are expecting cardioplegia to enhance myocardial aerobic metabolism. There are studies which shows that a cardioplegic heart consumes more oxygen while producing less lactate with different cardioplegic solutions [10,11]. In our study, with both techniques decrease in oxygen extraction (OE) following cross-clamping which does not rise to baseline values during rewarming indicates decreased oxygen metabolism during CPB. However reduced OE was not clinically notable. Lactate production, although within normal range, slightly increased during cross-clamping and rewarming with both myocardial protection methods. It is possible that decreased oxygen metabolism and a slight anaerobic condition caused this. Mrozinski et al. showed a lactate increment in favor of an anaerobic metabolism during off-pump CABG surgery under desflurane anesthesia [3]. Our research study does not cover all aspects of myocardial metabolism in detail

In our study enzyme levels (CK-MB) were comparable. At postoperative 6th and 24th hours Anderson et al. randomized 40 elective CABG operations into IAC or CCA groups and showed that CK-MB and troponin T levels were higher in cardioplegia group at 48th hour postoperatively. On the other hand, analysis of peak values and total enzyme levels showed no differences between the two groups. It was concluded that both of myocardial protection methods were effective in elective patients [2]. Gerola et al. performed a prospective randomized controlled trial in 60 elective CABG patients to compare the efficiency of IAC versus CCA with hot shot cardioplegic solution (Buckberg method) [5]. Hemodynamic parameters and CK-MB levels were similar and it was concluded that both methods protect the myocardium efficiently [5].

Even though Musumeci et al. revealed that peak serum troponin I, troponin T and CK-MB levels were higher in the CCA technique [12] there are other studies that support our findings. Gerola et al. and Alhan et al. found enzyme levels similar to our study [5,6]. Pepper et al. performed a prospective randomized trial which enrolled 50 patients to either CCA or IAC method for myocardial protection. Mean serum levels of myocardial enzymes were similar throughout postoperative 24 hours and analysis of left ventricular biopsy specimens showed that myocardial damage was similar in both groups [13]. Taggart et al. conducted a similar study with 20 patients in a prospective randomized trial comparing these two methods [14]. In this study, the duration of ischemia was (30 min and 32 min for CCA and IAC respectively) very similar to our study (31.6 min and 24.6 min for CCA and IAC groups, respectively) and they found that troponin T showed similar levels throughout postoperative 72 hours in both groups [14]. Cohen et

al. examined a small cohort of 24 consecutive CABG patients in a prospective randomized trial which compared these two techniques and levels of troponin T did not differ between the two groups, indicating similar protective properties [15]. Sunderdiek et al. conducted a prospective randomized trial in 103 consecutive CABG patients using either cold crystalloid cardioplegia (Bretschneider-HTK solution) or IAC and up to 10 days of postoperative monitorization of inotropic support, CK-MB and troponin I release and EKG changes did not demonstrated any significant differences between the groups [16].

According to the previous reports, the cross-clamping time was longer in cardioplegic arrest group but myocardial protection was similar in both techniques with respect to hemodynamic, and biochemical parameters and apoptotic changes in left ventricular biopsy specimens [2,6,16-19]. In our study, the duration of ischemia was similar in both groups, and myocardial protection was alike with both techniques.

Mild ischemia causes myocyte apoptosis, like in all other cells, while severe ischemia results in necrosis [17]. Apoptosis were shown in hibernating myocardial zone [18]. Ischemic preconditioning; reperfusion following short periods of ischemia, seems to play a role in reducing the permanent myocardial damage by preventing apoptosis [19,20]. In IAC method myocardium is technically exposed to ischemic preconditioning. Following every vascular distal anastomosis the myocardium was fibrillated by pace electrodes and the proximal anastomosis was made while the myocardium was reperfused. So we thought apoptosis would be more likely noticed in ICA method. However in both groups no apoptotic cell nuclei were detected. In this study, it seems that both myocardial protection techniques were equally effective with respect to apoptosis.

Limitations of study: We had to choose ASA II patients with good ventricular function because none of the surgeons were willing to take biopsy specimen from impaired ventricles. That is why the number of patients included in the study were relatively few; as this study is rather a preliminary human study.

There are few articles comparing these two protection methods by comparing oxygen extraction, lactate production and apopitosis formation during ischemia-reperfusion of the myocardial tissue.

In conclusion, the present study shows that for low risk CABG procedures, intermittent aortic cross-clamping with fibrillation and cardioplegic cardiac arrest methods were comparable to each other in protecting the myocardium from ischemic injury with respect to hemodynamic variables, myocardial oxygen extraction and lactate production and apoptosis in myocardial transmural biopsy specimens.

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