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Neural Protective Effect of Remote Ischemic Preconditioning During On-Pump Coronary Artery Bypass Graft

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Abstract

Background: Following cardiovascular surgery, remote ischemic preconditioning (RIPC) might lessen ischemic injury. On clinical results, RIPC might have an advantage, however this is not certain. Our study aimed to improve the outcome of on-pump CABG patients (cardiac events, ICU stay & inward stay. **Patients and methods**: We conducted a blinded randomised controlled trial to ascertain if RIPC led to fewer negative clinical outcomes following heart surgery. We compared the neural protective effect of RIPC in patients undergoing on-pump CABG using serum level of S100B protein, MMSE score and CAM test to assess the postoperative cognitive dysfunction. The intervention involved applying RIPC to the upper limb for 4 cycles of 5 minutes each, followed by 5 minutes of rest. The control group received a false intervention. Patients were recruited from among those undergoing coronary artery bypass graft and at high risk of ischemic complications. Mortality, myocardial infarction, stroke, renal failure, respiratory failure, and low cardiac output syndrome made up the major end point. The separate outcome characteristics that went into this score were the secondary end points. **Results**: The composite outcome (RIPC: 67 [32%] and control: 72 [34%], relative risk [0.94 0.72-1.24]), and the individual elements that made up the composite outcome, were the same for the 2 groups. **Conclusion**: We found no differences in the length of hospital stays overall or in the intensive care unit across the groups. RIPC did not improve the clinical results for patients who had undergone cardiac surgery.

Keywords: Cardiovascular Surgery; Ischemia Injury; Remote Ischemic Preconditioning.

Introduction

Cardiac surgery is a success of contemporary medicine, and it is widely regarde d as a safe treatment. Unfortunately, postoperative cognitive problems are still an issu e. After undergoing cardiac surgery, up to 40:60% of patients may experience postope rative cognitive dysfunction (POCD) **(1)**.When assessed within two weeks of surgery, this percentage drops to 30–40%, rises to 10–20% at one year, and then increases again at three–five years. 65% of patients are discharged from hospitals **(2)**.

Remote ischemic preconditioning (RIPC) is a mechanism wherein cells develop resistance to ischemia-reperfusion (I/R) injury when subjected to regulated periods of transient, sub-lethal ischemia prior to a lengthy ischemia **(3)**. It protects against IR harm in an organ at a remote site **(4)**.

Cardiopulmonary bypass (CPB) during cardiac surgery causes ischemia-reperfusion (I/R) harm to a number of organs **(5)**. A potent phenomenon (RIPC) enables the protection of the myocardium against I/R injury by temporary, non-damaging I/R episodes administered to an organ located outside of the heart **(6,7)**.

The ability to non-invasively create RIPC stimulus by simply inflating and deflating a blood pressure cuff over systemic blood pressure has made it easier to apply it in a therapeutic environment. RIPC has become a popular method to lessen myocardial damage and enhance patient outcomes after cardiac surgery **(8)**. However, it is uncertain if RIPC shields patients having coronary artery bypass graft (CABG) surgery from myocardial damage. One of the most common cardiac surgical procedures, CABG surgery, has insufficient evidence particularly addressing RIPC. Additionally, there is conflicting information regarding RIPC-induced renal protection in relation to cardiac surgery.

Therefore, we decided to conduct a trial of RIPC in CABG surgery to see if it would have any neural protective effects in those patients undergoing CABG.

Patients and methods

This study was a prospective randomized comparative clinical trial. The study was taken place at Suez Canal University Hospitals in the scheduled operating theatres since March 2017 till July 2019. The study included 40 patients undergoing elective on-pump CABG. we excluded patients who have previously documented cognitive deficits, cerebrovascular accident Upper extremity vascular disease redo CABG surgery and carotid significant atherosclerosis.

Patients with chronic renal, severe hepatic insufficiency and patients on medications altering cognitive functions as pregabalin also excluded.

After obtaining approval of the Research Ethics Committee of the Faculty of Medicine, Suez Canal University, and a signed written informed patient consent explaining the purpose, effects, technique and complications, patients were recruited and randomly assigned into one of two equal groups on alternative basis.

Group 1 (preconditioning group): for 20 patients, remote ischemia preconditioning was used (RIPC) Using a tourniquet inflated to 100 mmHg over systolic blood pressure and 4 cycles of brief (5 minutes) left upper extremity ischemia, followed by 5-minute reperfusion periods during which the tourniquet would be deflated, RIPC was achieved in a subset of individuals. Before starting cardiopulmonary bypass and cannulating the aorta, repetitive short occlusion and reperfusion was finished.

Group 2 (control group): Twenty patients underwent conventional intraoperative care. Standard intraoperative management: All patients were given a tourniquet that was applied to their left upper extremity while still deflated.

Surgical and anesthetic techniques:

All patients received as a premedication IV midazolam (0.05 mg/kg) and morphine (0.01mg/kg) after insertion of wide-pore intravenous catheter in the right arm. Intra-arterial monitoring via the right radial artery was established. Monitoring equipment's (Datex-Ohmeda™) were used including 3 leads ECG, invasive blood pressure, pulse oximeter, capnograph and central venous line after intubation.

The depth of anesthesia was monitored with bispectral index device. The target BIS range was 40-60 for surgical anesthesia. patients received intravenous fluid according to the need of each patient and guided CVP.

After a minimum of three minutes of pre-oxygenation with 100% oxygen, anaesthesia was induced using a combination of propofol (1-2 mg/kg), cis-atracurium (0.15 mg/kg), and fentanyl (3-5 mcg/kg). The patients were then intubated with an endotracheal tube of the proper size and ventilated with 100% oxygen. Isoflurane was used to maintain anaesthesia, with its end tidal concentration varied to maintain BIS between 60 and 40% while oxygen flow rate was maintained at 2 litres per minute in a totally closed circuit with co2 absorbent and cis-atracurium 0.03 mg/kg guided neuromuscular monitor (TOF). Hemodynamics (heart rate and mean arterial blood pressure) remained within 25% of baseline values.

Every surgical procedure was carried out using the midline sternotomy approach. An anesthesiologist administered full heparinization with the intention of achieving an active clotting time of at least 480.

The patient was put on cardiopulmonary bypass and given topical cold ice slush-delivered clold blood crystalloid antegrade cardioplegia. Throughout the cross-clamp period, the patient's body temperature fell to 30-32 degrees Celsius. Standard procedures were used to execute a coronary artery bypass graft on the pump using harvested saphenous vein and left internal mammary artery grafts. Aortic cross clamp was unfastened following CABG. Temporary pacing wires were positioned according to protocol. The patient was weaned from cardiopulmonary bypass after an appropriate reperfusion interval and gradual rewarming. The patient received proton pump inhibitor and was decannulated (Dosage for heparin reversal is 1.0 -to- 1.5 mg protamine sulphate IV for every 100 IU of active heparin).

After adequate hemostasis achieved, accepted hemodynamics patient was transferred to the cardiac surgery intensive care unit. Neural injury was assessed by measuring serum S100B protein level before induction of anesthesia (Pre), at postoperative Day 1 (Post 1) and at postoperative day 7 (Post 7).

The incidence of short term postoperative cognitive dysfunctions (POCD) was assessed by using the Mini Mental State Examination (MMSE) and the incidence of delirium Confusion Assessment Method (CAM), in day 1 and day 7 postoperative.

Measurements:

1- **Biomarker of neural injury (serum S100 B)**

Collecting peripheral blood samples withdrawn from the central venous catheter. In a tube with a clot activator (CAPIJECT® T-MG; Terumo Medical Co., Somerset, NJ, USA), all samples were put. Using an ELISA kit, serum S100B concentrations were determined (ab234573 S100B Simple Step ELISA®, © 2018 Abcam Co, UK).

2- Postoperative cognitive dysfunction (POCD)

The incidence of delirium and short term postoperative cognitive dysfunctions (POCD) by using mini mental state examination (MMSE) The MMSE[(91)] is a 20-item screening test that measures cognitive impairments. The maximum **MMSE** score is

30 points. A score of less than 24 out of 30 is used as the screening threshold to indicate cognitive impairment.

A systematic, evidence-based screening method called the Confusion Assessment Method (CAM) enables practitioners without psychiatric training to swiftly and accurately identify and characterise delirium in both clinical and research settings. We used (CAM-ICU) flow sheet for assessment and recorded on day 1 and day 7 postoperative.

3- Perioperative adverse cardiac events

- Perioperative mortality was included all-cause intraoperative and postoperative mortality to 7 days
- Incidence of stroke, atrial fibrillation (AF) or need for renal dialysis (when not on dialysis preoperatively)

4- Hospital-stay in days

Length of post-operative hospital stay (ICU and inward) in days was recorded for both groups

Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics®22. Descriptive data was expressed as median and interquartile range (-) for continuous nonparametric variables, as mean and SD for continuous parametric variables, and count/total and percentages (%) for categorical and dichotomous variables. Student T-test was used to analyze the continuous variables between the two studied groups (i.e., hospital stay) and Chi-test for categorical and dichotomous variables (mortality, incidence of clinical events). One-way analysis of variance (ANOVA) with Repeated Measures was used to analyze the continuous variables among the follow-up points within the same group. Statistically significant differences among the different readings were further assessed using Fisher's least significant difference (LSD) post hoc analysis. The level of statistical significance was considered to be $p<0.05$. Presentation of the statistical outcomes in the form of tables and graphs was performed using the "Microsoft Office Excel® 2007" program.

Results

Regarding demographic (e.g., age, sex, BMI) and clinical (ASA physical status, comorbidities and number of diseased vessels) characteristics both groups were matched **(Table 1)**.

Procedure characteristics among both groups shows that both bypass and cross clamp durations, total volume of cardioplegic solution, the time taken to regain normal sinus rhythm, incidence of reperfusion arrhythmia and need for cardioversion were non-significantly different between both groups **(Table 2)**.

Graph (1) and (2) shows that there were non-significant differences in mean arterial blood pressure and Intraoperative heart rate between both groups at all-time intervals.

Table (1): Demographic and clinical characteristics among both groups

		Preconditioning group (n=20)	Control group (n=20)	P value
Age		**57.0 ± 6.6**	**58.3 ± 7.5**	**0.582(NS)**
Male/Female		**12/8**	**13/7**	**0.744(NS)**
Height (m)		**1.77 ± 0.047**	**1.75 ± 0.044**	**0.136(NS)**
Weight (kg)		**90.3 ± 6.4**	**90.7 ± 7.5**	**0.840(NS)**
BMI (kg/m^2)		**28.8 ± 2.9**	**29.82 ± 3.1**	**0.354(NS)**
ASA (II/III)		**9/11**	**10/10**	**0.752(NS)**
Diabetes		**12 (60%)**	**9 (45%)**	**0.342(NS)**
Hypertension		**14 (70%)**	**13 (65%)**	**0.736(NS)**
Smoking		**5 (25%)**	**8 (40%)**	**0.311(NS)**
No. of diseased vessels	**1** **2** **3** **4**	**1 (5%)** **7 (35%)** **11 (55%)** **1 (5%)**	**2 (10%)** **9 (45%)** **8 (40%)** **1 (5%)**	**0.787(NS)**

Data are presented as mean ± standard deviation, count/count and number (%), * statistically significant difference (P value < 0.05), NS: non-significant difference (P value > 0.05)

Table (2): Procedure characteristics

	Preconditioning group (n=20)	Control group (n=20)	P value
CPB duration (min)	**77.9 ± 6.7**	**76.4 ± 8.1**	**0.542(NS)**
Aortic cross clamp duration (min)	**41.3 ± 9.2**	**39.1 ± 10.3**	**0.481(NS)**
Time to regain NSR	**6.2 ± 1.7**	**6.9 ± 1.8**	**0.222(NS)**
Volume of cardioplegic solution (ml)	**1275.1 ± 302.4**	**1187.5 ± 342.9**	**0.650(NS)**
Reperfusion arrhythmia	**2 (10%)**	**4 (20%)**	**0.376(NS)**
DC need	**1 (5%)**	**4 (20%)**	**0.151(NS)**

Data are presented as mean ± standard deviation and number (%), CBP: cardiopulmonary pump, NSR: normal sinus rhythm, * statistically significant difference (P value < 0.05), NS: non-significant difference (P value > 0.05).

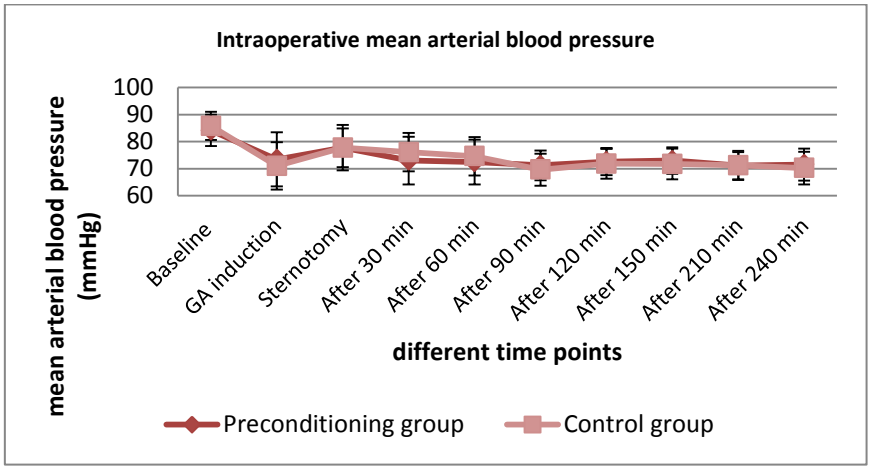

Fig. (1): Intraoperative mean arterial blood pressure (mmHg) at different time points among both groups.

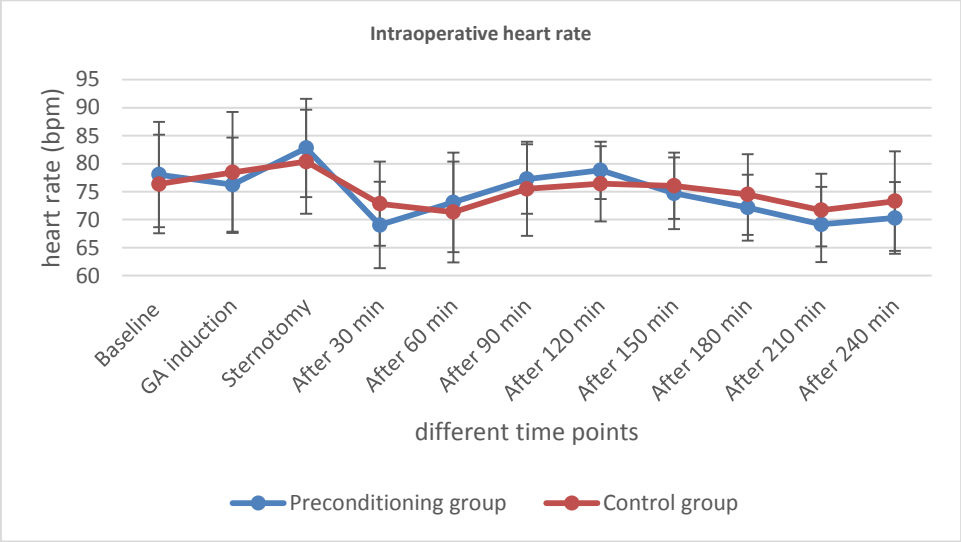

Fig (2): Intraoperative heart rate (beat per min) at different time points among both groups.

Laboratory biomarkers of brain injury (S100β ng/ml)

Despite being non-significantly different at baseline (T0), serum S100β level was significantly lower in the Preconditioning group than in the control group in the first postoperative day (T1). In addition, the rise in the first day (T0-T1) was also significantly higher in the control group compared to the Preconditioning group. On the other hand, serum S100β level was non-significantly different between both groups in the seventh postoperative day (T2), while the rise in the first day (T0-T2) was also non-significantly different between both groups. Moreover, S100β level significantly increased in the first postoperative day compared to baseline within both groups. However, the third sample (T2) was non-significantly different within both groups compared to baseline; **Table (3).**

Table 3: Laboratory biomarkers of brain injury (S100β ng/ml) among both groups at different endpoints

	Preconditioning group (n=20)	Control group (n=20)	P value
Baseline (T0)	**0.37 ± 0.09**	**0.36 ± 0.10**	**0.672(NS)**
After 24 hours (T1)	**0.88 ± 0.66$^{\neq}$**	**2.48 ± 1.22$^{\neq}$**	**0.000***
Change (T0-T1)	**0.50 ± 0.68**	**2.12 ± 1.24**	**0.000***
After 7 days (T2)	**0.35 ± 0.11**	**0.51 ± 0.35**	**0.061(NS)**
Change (T0-T2)	**0.09 ± 0.06**	**0.21 ± 0.31**	**0.103(NS)**

Data are presented as mean ± standard deviation, * statistically significant difference between both groups (P value < 0.05), NS: non-significant difference (P value > 0.05) between both groups, $^{\neq}$ statistically significant difference compared to baseline reading within same group

Table (4) shows that in the first postoperative day, 4 patients (20%) in the control group developed postoperative delirium according to CAM test, compared to only one patient in the Preconditioning group. In the seventh postoperative day, only 2 patients (10%) in the control group still had delirium. However, such differences were statistically non-significant between both groups. despite being non-significantly

different at baseline, MMSE score was significantly higher in the Preconditioning group than in the control group in the first postoperative day. In addition, the decline in the first day compared to baseline was also significant in both groups. On the other hand, MMSE score was non-significantly different between both groups in the seventh postoperative day, but the decline in the seventh day compared to baseline was still significant only in the control group.

Table 4: postoperative cognitive dysfunctions (POCD) by using mini mental state examination (MMSE) Confusion Assessment Method (CAM)

		Preconditioning group (n=20)	Control group (n=20)	P value
Delirium	Day 1	1 (5%)	4 (20%)	0.147(NS)
	Day 7	0	2 (10%)	0.432(NS)
MMSE score	Baseline	27.83 ± 1.94	28.33 ± 1.21	0.599 (NS)
	Day 1	22.43 ± 2.43$^{\neq}$	19.17 ± 2.14$^{\neq}$	0.023*
	Day 7	26.67 ± 3.52	24.58 ± 3.39$^{\neq}$	0.063 (NS)

Data are presented as number (%), mini mental state examination (MMSE). Data are presented as mean ± standard deviation, * statistically significant difference between both groups (P value < 0.05), NS: non-significant difference (P value > 0.05) between both groups, $^{\neq}$ statistically significant difference compared to baseline reading within same group.

All general postoperative outcomes including; the adverse cardiac events and ICU, inpatient and hospital stay were non-significantly different between both groups **(Table 5).**

Table (5): General postoperative outcomes among both groups

		Preconditioning group (n=20)	Control group (n=20)	P value
Adverse cardiac events	Death	0	0	-
	Postoperative MI/CHF	0	0	-
	Stroke	0	0	-
	Need for IABP	0	0	-
Postoperative stay	ICU stay (days)	2.70 ± 0.47	2.85 ± 1.22	0.612(NS)
	Inward stay (days)	5.55 ± 0.89	5.70 ± 0.98	0.614(NS)
	Hospital-stay (days)	8.25 ± 1.16	8.55 ± 1.96	0.560(NS)

Data are presented as number (%), POD: postoperative day, NS: non-significant difference (P value > 0.05) between both groups

DISCUSSION

A future protracted episode of ischemia and reperfusion can harm an unrelated tissue, although ischemia preconditioning of one tissue can shield it from this harm. The brain, heart, kidney, liver, stomach, pancreas, and skeletal muscle have all shown evidence of this amazing "remote ischemic preconditioning" (RIPC) transfer of protection from one preconditioned tissue to another. The most frequent method of producing RIPC is brief, repeated ischemia of the upper or lower extremities **(9).**

RIPC has been demonstrated to improve cortical neuronal activity recovery, maintain cerebral oxygen tension, and lessen cortical damage following hypothermic circulatory stoppage. These findings support the intriguing hypothesis that RIPC could be helpful for reducing the frequency and severity of perioperative strokes or for minimising the more subtle alterations in neurologic function (such as delirium and cognitive impairment) that are frequently noticed in patients undergoing cardiac surgery using cardiopulmonary bypass **(6)**.

In our study, both groups were matched regarding demographic (e.g., age, gender, BMI) and clinical (ASA physical status, comorbidities and number of diseased vessels) characteristics. Regarding our primary outcome, we found that despite being non-significantly different at baseline, serum S100β level was significantly lower in the Preconditioning group than in the control group in the first postoperative day. In addition, the rise in the first day was also significantly higher in the control group compared to the Preconditioning group. On the other hand, serum S100β level was non-significantly different between both groups in the seventh postoperative day, while the rise in the first day was also non-significantly different between both groups.

In concordance with our study, **Jing and Zheng (10)** performed a study on forty patients undergoing cardiac valve replacement with CPB and randomly divided them into two groups: control group (C) and experiment group (E), each having 20 cases. In E group, remote ischemic preconditioning (RIPC) was induced by four cycles of ischemia and reperfusion on the left upper arm using a blood pressure cuff. They found that in both groups, the release of S-100β protein became higher than that before induction at T2, reached the peak level at T3, and began to decrease at T4. In E group, the levels of serum S-100β at time points T2-T6 were significantly lower than those in C group ($P<0.05$).

Regarding RIPC potentiality for cerebral protection, similar results were reported by **Jensen et al. (11)**. They randomized twelve piglets to control and RIPC groups. Prior to cardiopulmonary bypass, RIPC was induced using 4 rounds of hind limb ischemia for 5 minutes each. Cardiopulmonary bypass was performed on all animals, and then HCA at 18°C for 60 minutes was performed. They found that brain lactate concentration was significantly lower and recovery of electroencephalographic activity faster in the RIPC group. RIPC had a beneficial effect on neurological function during the 7-day follow-up.

In contrast to our study, **Lucchinetti et al**. **(8)**, conducted a placebo-controlled randomized controlled study that included fifty-five elective on-pump CABG patients. Patients in the RIPC group received four 5-min cycles of 300mmHg cuff inflation/deflation of the leg before aortic cross-clamping. They found that biomarker for the cerebral injury (S100) did not show protection with RIPC in isoflurane-anesthetized patients **(8)**. This could be explained by the fact that they used propofol/sufentanil for anesthesia maintenance, while we used isoflurane/fentanyl in our study .

Moreover**, Zhong et al**. **(12)** investigated the cardio- cerebral protective effects of remote ischemic post-conditioning (RPostC) on children undergoing open- heart surgery for repair of congenital heart defects (CHD). They found that postoperative concentrations of S100β were not significantly different between both groups **(12)**. This could be explained by the fact that they applied remote ischemic post-conditioning rather than preconditioning (i.e., RIPostC was induced by three 5- min cycles of lower limb ischemia and reperfusion using a blood pressure cuff (200 mmHg) at the onset of aortic unclamping), while we completed RIPC before aortic cannulation and initiation of cardiopulmonary bypass. This idea was supported by the study published by **Gao et al**. **(13)**. They conducted an experimental protocol where focal ischemia was produced by permanent occlusion of the cerebral artery combined with 30 min of occlusion of both common carotid arteries in male rats, they were able to compare post conditioning's protection with that of both rapid and delayed preconditioning. They discovered that infarct sizes were decreased by both rapid and delayed preconditioning carried out 60 minutes and 3 days prior to stroke. However, when postconditioning was paired with either a quick or a delayed preconditioning, no additional protection was found **(13)**.

In the current study, we also found that both bypass and cross clamp durations, total volume of cardioplegic solution, the time taken to regain normal sinus rhythm, incidence of reperfusion arrhythmia and need for cardioversion were non-significantly different between both groups. Moreover, that there were non-significant differences in mean arterial blood pressure or heart rate between both groups at all time intervals. Similar findings were also reported by **Jin et al. (14)** and **Cheung et al (15)**.

In our study, we reported that in the first postoperative day, 4 patients (20%) in the control group developed postoperative delirium according to CAM test, compared to only one patient in the Preconditioning group. In the seventh postoperative day, only 2 patients (10%) in the control group still had delirium. However, such differences were statistically non-significant between both groups. In addition, we found that despite being non-significantly different at baseline, MMSE score was significantly higher in the Preconditioning group than in the control group in the first postoperative day. In addition, the decline in the first day compared to baseline was also significant in both groups. On the other hand, MMSE score was non-significantly different between both groups in the seventh postoperative day, but the decline in the seventh day compared to baseline was still significant only in the control group.

In agreement with our findings, a study conducted by **Hudetz et al. (6)** investigated 15 nonsurgical individuals served as controls whereas 30 men underwent elective coronary artery or valve surgery while utilising CPB. They discovered no differences between the RIPC and control groups in the prevalence of delirium.

In addition, **Meybohm et al**. **(16)** conducted 180 adult patients who were receiving cardiopulmonary bypass as part of elective heart surgery were included in RIPC. Patients were randomly assigned to the control group or the RIPC. They failed to show that a RIPC approach is effective in reducing the frequency and severity of POCD. Similarly**, Meybohm et al**. **(17)** compared upper-limb RIPC to a control

group in people scheduled for elective heart surgery requiring cardiopulmonary bypass under general anaesthesia with intravenous propofol.

No significant differences between the RIPC group and the sham-RIPC group were seen in the incidence of postoperative delirium (17)

In our study, we reported that all general postoperative outcomes including; the adverse cardiac events and ICU, inpatient and hospital stay were non-significantly different between both groups .

In concordance with our study, The Remote Preconditioning **Trialists' Group (18)** conducted a review that identified several randomised clinical studies using RIPC that served as "proof-of-concept" (23 trials of RIPC in 2200 patients undergoing major adult cardiovascular surgery). They discovered that the clinical endpoints (death, peri-operative myocardial infarction (MI), stroke, hospital or critical care length of stay) were not significantly affected by RIPC. Pilot study data combined couldn't prove that RIPC had any substantial impact on clinically important endpoints.

Another systematic review and subsequent meta-analysis of randomized controlled trials of RIPC versus usual care (control group) was performed by **King et al. (19)**. Eighteen studies, totaling 4551 participants were analyzed. They found no significant difference between RIPC and control when mortality, the incidence of new onset atrial fibrillation, intensive care unit stay in days and hospital stay in days were compared.

Similarly, **Meybohm et al. (17)** found no significant between-group difference in death, myocardial infarction, and stroke. The length of mechanical ventilation, the amount of time spent in the hospital or intensive care unit, and the onset of atrial fibrillation for the first time were not significantly different between the RIPC group and the control group.

Conclusion

A novel non-invasive therapeutic method has been made available by RIPC to stop acute cerebral I/R injury. As we found that S100B protein (which is an indication of cerebral affection) significantly lower in the RIPC group in the 1st day postoperative which may indicate cerebral protection. However, we reported no significant difference between both techniques as regard incidence postoperative cognitive dysfunction and delirium, all general postoperative outcomes including; the adverse cardiac events and ICU, inpatient and hospital stay.

No Conflict of interest.

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