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ORIGINAL INVESTIGATION

Effect of mechanical ventilation during cardiopulmonary bypass on oxidative stress: a randomized clinical trial

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KEYWORDS
Oxidative stress;
Peep;
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Abstract
Background: Cardiopulmonary bypass (CPB) causes systemic oxidative stress response and endothelial damage in systemic organs. We investigated the effects of positive end-expiratory pressure (PEEP) and mechanical ventilation (MV) applications on oxidative stress in CPB.
Methods: Seventy-one patients were recruited and 60 completed the study. Randomized groups: MV off (Group 1); MV on, tidal volume (TV) at 3–4 mL.kg^{-1} (Group 2); MV on, TV at 3–4 mL.kg^{-1}, PEEP at 5 cmH2O (Group 3), n = 20 in each group. As oxidative stress markers, we used glutathione peroxidase (GPx), total antioxidant status (TAS), total oxidant status (TOS), total and native thiol (TT, NT), malondialdehyde (MDA), and catalase. We also investigated the correlation between oxidative stress and postoperative intubation time.
Results: The postoperative GPx levels in Group 2 were higher than Group 3 ($p = 0.017$). In groups 2 and 3, TAS levels were higher postoperatively than intraoperatively ($p = 0.001$, $p = 0.019$, respectively). In Group 2, the TT levels were higher postoperatively than preoperatively and intraoperatively ($p = 0.008$). In Group 3, the postoperative MDA levels were higher than preoperatively ($p = 0.001$) and were higher than both postoperative levels of Group 1 and 2 ($p = 0.043$, $p = 0.003$). As the preoperative TAS (Group 2) decreased and the postoperative NT (Group 2) and catalase (Group 3) increased, the postoperative intubation time lengthened.
Conclusion: MV (3–4 mL.kg^{-1}) alone seems to be the most advantageous strategy. Prolonged postoperative intubation time was associated with both increased NT and catalase levels.
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Introduction

Cardiopulmonary bypass (CPB) can result in serious functional changes in the organs of patients and even complex and non-physiological conditions.[1–3] CPB plays an important role in determining lung damage. Several factors contribute to this injury: atelectasis, hyperoxygenation that causes free radicals to be released, and the systemic inflammatory response associated with CPB.[4] It is common practice to stop ventilation during CPB because lung function is performed by an extracorporeal gas exchanger. However, interruption of mechanical ventilation (MV) during CPB is associated with the development of microatelectasis, hydrostatic pulmonary edema, and decreases both lung compliance and surfactant diffusion.[5] Lung-sparing ventilator strategies, including low tidal volume (TV), continuous positive airway pressure (CPAP), and higher positive end-expiratory pressure (PEEP) levels, may help reduce postoperative pulmonary complications and inflammation.[6] Aortic cross-clamp in CPB can result in ischemia-reperfusion injury during surgery. This injury causes substantial myocardial stress, thereby inducing the formation of reactive oxygen species (ROS) and pro-inflammatory mediators that damage proteins, lipids, and DNA; this damage affects postoperative cardiac functions and outcomes.[7] ROS strongly contributes to reperfusion injury.[8,9] Free radicals are formed at a maximum of 3 to 5 minutes of reperfusion and are present for 3 hours,[10,11] indicating they are an important factor in myocardial depression.[12,13] Malondialdehyde (MDA) is the most commonly used lipid peroxidation product and is a widely used indicator of oxidative stress. Lipid peroxidation can cause both decreases in membrane viscosity and permeability and membrane protein denaturation. Glutathione peroxidase (GPx) and catalase (CAT) are endogenous antioxidant markers.[14] Total oxidant status (TOS) and total antioxidant status (TAS) project the redox balance between second oxidation and antioxidation. TOS measurement is an indicator of ROS while TAS is an indicator of all antioxidants.[15] Thiols are sulfur group-having compounds, which are crucial antioxidant buffers that interrelate with physiologic oxidants.[16]

Different studies have been conducted on lung-protective mechanisms such as different PEEP and tidal volume.[10,12]

This study aimed to investigate the effects of various mechanical ventilation applications on MDA, TOS, TAS, GPx, CAT, total thiol (TT), native thiol (NT) and to analyze the correlation between oxidative stress parameters and postoperative intubation time.

Methods

Study population

The faculty's Clinical Research Ethics Committee approved this study (2018/10-12). The study was conducted following the principles of the Declaration of Helsinki. The trial is registered at clinicaltrials.gov (NCT03601364). All patients were individually provided written informed consent. The study was conducted between August 2018 and March 2019. Patients with American Society of Anesthesiologists (ASA) physical status III–IV were included if they were conscious, aged 18–80 years, did not require emergency surgery, agreed to participate in the study, underwent CPB, and were extubated in the first 24 postoperative hours. Patients were excluded if they had acute coronary syndrome, were in emergency status, had acute myocardial infarctions in the previous month, had off-pump coronary artery bypass, had chronic inflammatory diseases (e.g., rheumatoid arthritis and psoriasis) or autoimmune diseases, were taking immune suppressive medication, had liver disease or chronic or acute renal failure, or had active infections.

Study design

This prospective, randomized, controlled study was conducted on 71 patients to investigate the effects of positive end-expiratory pressure (PEEP) and mechanical ventilation on oxidative stress in CPB. Age, sex, BMI, smoking, comorbidities, types of surgery, left ventricle E/aF, CPB duration (min), cross-clamp time (min), operation time, postoperative intubation time (min), and ICU stay (days) have been recorded. Fluid intake and urine output were recorded intraoperatively. The primary outcomes were the effects of different mechanical ventilation strategies of the groups on both intraoperative and postoperative oxidative stress parameters. The secondary outcomes were to evaluate the oxidative stress parameters within the group, as well as the correlation between oxidative stress levels and postoperative intubation time.

Closed opaque envelopes were delivered to the patients considering their group assignments in the patient room. The randomized 60 patients were randomly divided into three groups as follows. Group 1 (n = 20): mechanical ventilator off. Group 2 (n = 20): mechanical ventilator on, TV at 3–4 $mL.kg^{-1}$, FiO_2 at 50%, flow at 2 $L.min^{-1}$, and frequency at 10–12. Group 3 (n = 20): mechanical ventilator on, TV at 3–4 $mL.kg^{-1}$, PEEP at 5 cm H_2O, FiO_2 at 50%, flow at 2 $L.min^{-1}$, and frequency at 10–12. This study has incorporated GPX, catalase, TAS, and thiols as antioxidants while MDA and TOS as oxidative stress markers.

Anesthesia and cardiopulmonary bypass management

The same surgery and anesthesia team performed all the operations. The patients underwent radial artery catheterization and were monitored. Anesthesia was induced using midazolam (Zolamid®, Defarma, Tekirdag, Turkey) (0.1 $mg.kg^{-1}$, intravenously), fentanyl (Talinat®, Vem, Istanbul, Turkey) (5–8 $\mu g.kg^{-1}$, intravenously), and rocuronium bromide (Myocron®, Vem, Istanbul, Turkey) (0.6 $mg.kg^{-1}$, intravenously). Sevoflurane (Sevorane®, Abbvie, Istanbul, Turkey) was used during general anesthesia. For maintenance of intraoperative anesthesia, Primus was used as an anesthetic machine (Drager, Lübeck, Germany). Rocuronium bromide (0.6 $mg.kg^{-1}$) was administered every 30 minutes. Every patient underwent a median sternotomy. Heparin was administered at 300–500 $units.kg^{-1}$. Whole blood cardioplegia and del Nido cardioplegia were used. Throughout the procedure, an activated clotting time > 400 seconds and a mean arterial pressure > 60 mmHg were maintained. Heparin was neutralized with 1–1.3 mg of protamine sulfate.

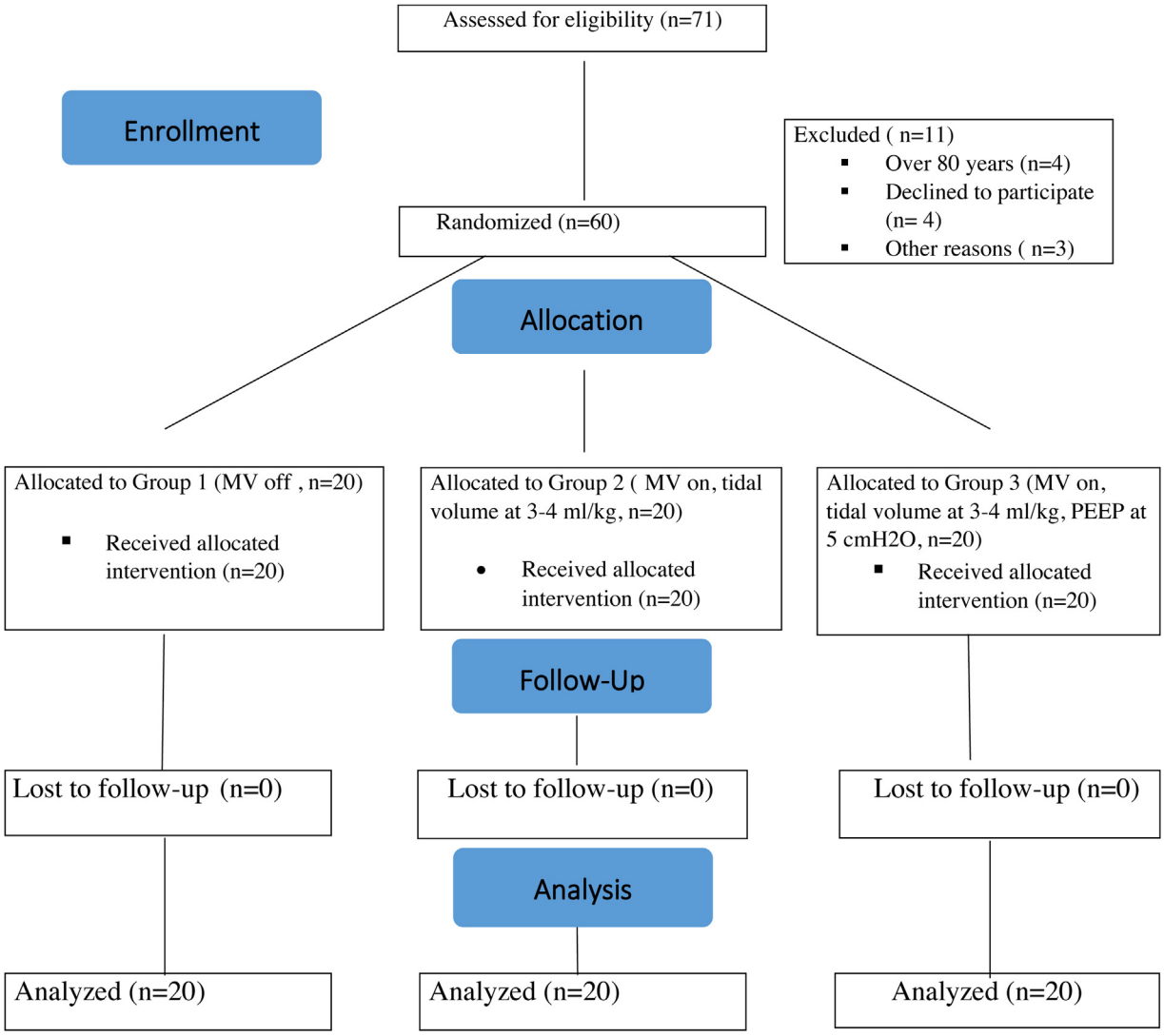

Figure 1 Consort diagram of the study.

CPB was managed with a roller pump with a membrane oxygenator (Stockert, Sorin Group, München, Germany) and arterial line filter at pump flow rates of 2–2.4 $L.min^{-1}.m^{-2}$. Standard CPB was applied with mild hypothermia (32 °C). Following the surgery, every patient was transferred to the cardiovascular surgery intensive care unit (ICU).

Sample collection

For all patients, the preoperative blood samples were taken after an arterial cannula was inserted. The intraoperative blood samples were collected from the radial artery 3–5 minutes after removing the cross-clamp. The postoperative blood samples were collected at the 24th hour in ICU.

The heparinized blood samples were sent to the laboratory under suitable conditions and centrifuged at 3,000 rpm for 5 minutes to separate the plasma. The plasma samples were thrice-diluted using a physiological saline solution and stored in a deep freezer at −80 °C before biochemical analysis.

Oxidative stress analysis

Malondialdehyde: minute changes in the concentration of serum lipid peroxidation (total MDA) were identified using previous methods[17] and are expressed as nanomoles per milliliter ($nmol.mL^{-1}$).

Glutathione peroxidase: the Beutler method was used. GPx catalyzes the oxidation of reduced glutathione (GSH) into oxidized GSH (glutathione disulfide, GSSG) via H_2O_2. In the presence of H_2O_2 or *t*-butyl hydroperoxide, the GSSG formed by GPx is reduced to GSH via GSH reductase and NADPH. The GPx activity was determined by spectrophotometrically reading the absorbance difference of NADPH at 340 nm during its oxidation to NADP.[18]

Catalase: we measured the H_2O_2 degradation rate using the Beutler method. The disappearance rate of H_2O_2 was monitored spectrophotometrically at 230 nm. The assay

medium comprised 50 μl of 1 mol Tris-HCl buffer (pH 8), 930 μl of 10 mmol H_2O_2, 930 μl of deionized water, and 20 μl of serum sample. One unit of CAT in serum, expressed as 1 $U.mL^{-1}$, is the amount of enzyme required to destroy approximately 90% of the substrate in 1 mL within 1 minute.[19]

Total antioxidant status: the TAS levels were measured using Rel Assay commercial kits (Rel Assay Kit Diagnostics, Turkey) with a spectrophotometric method. Trolox, which is a water-soluble analog of vitamin E, was used as the calibrator. The results are expressed as mmol Trolox equiv./lt.[20]

Total oxidant status: the TOS levels were determined using Rel Assay commercial kits (Rel Assay Kit Diagnostics, Turkey) with a spectrophotometric method. The calibrator used was H_2O_2. The results are expressed as μmol H_2O_2 equiv./lt.[20]

Thiol measurement: Serum NT and TT ($\mu mol.L^{-1}$) were evaluated as described previously.[21] Primarily, disulfide bonds were reduced to functional thiol groups in the presence of sodium borohydride, which was then removed with formaldehyde. All reduced or non-reduced thiol groups were evaluated via the 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) reaction. Thereafter, we calculated half of the difference between the TT and NT values and the dynamic disulfide amounts.

Statistical analysis

In this study, a power analysis was applied to determine the sample sizes of the groups in the study.[22]

Power analysis was performed by considering the TAS values (Group T: 1.29 ± 0.30 and Group C: 1.04 ± 0.18) in the reference study[22] at the type I error level with α: 0.05, β: 0.20 type II error level and 0.80 power of the test. As a consequence of this power analysis, it was planned to involve 51 individuals from three groups of 17. We included a total of 60 patients in this study, including 20 patients in each group.

In the data analysis, the variables were assessed for a normal distribution with the Shapiro-Wilk test. The non-normally distributed variables were compared between groups using the Kruskal-Wallis H test. For multiple comparisons, the Bonferroni and Dunn-Sidak tests were performed. The normally distributed variables were compared by one-way ANOVA. We used Tukey's HSD and Tamhane's T2 tests for multiple comparisons, and we employed chi-square and exact tests for the analysis of categorical variables. For the differences between repeated measurements in non-normally distributed variables, we used the Friedman test. Likewise, the Bonferroni and Dunn-Sidak tests were performed for multiple comparisons. For the relationship between variables, the Spearman correlation test was utilized. Statistical significance was defined as $p \leq 0.05$. The data were evaluated using IBM SPSS version 22 (IBM Corp., Armonk, NY, USA).

Results

Seventy-one patients were assessed for eligibility. Eleven patients were excluded from the study and 60 patients were included in the study after randomization. All patients completed the study (Fig. 1). Hypertension was significantly higher in Group 1 than the other groups ($p = 0.015$) (Table 1). Operation time was longer in Group 2 than both Group 1 and Group 3 (Table 2).

Primary outcomes

The preoperative, intraoperative, and postoperative TAS levels were higher in Group 3 than Groups 1 and 2 ($p < 0.001$). The preoperative and postoperative GPx levels of Group 2 were higher than Group 3 ($p = 0.024$ and $p = 0.017$, respectively). In the intraoperative period, the TT levels of Groups 1 and 2 were higher than Group 3 ($p = 0.017$). In the preoperative period, the TOS level of Group 3 was higher than Group 2 ($p = 0.012$). However, in the intraoperative period, the TOS level of Group 2 was higher than Groups 1 and 3 ($p < 0.001$). The TOS levels were higher postoperatively in Groups 2 and 3 than Group 1 ($p < 0.001$). While the preoperative MDA level was higher in Group 1 than Group 3, the intraoperative and postoperative MDA levels were higher in Group 3 than Groups 1 and 2 ($p = 0.003$ and $p < 0.001$, respectively) (Table 3).

Secondary outcomes

In Groups 2 and 3, the postoperative TAS levels were higher than the intraoperative TAS levels ($p < 0.001$ and $p < 0.019$, respectively). In Group 1, the postoperative CAT value was higher than the preoperative CAT level ($p = 0.001$). The intraoperative CAT level of Group 2 was higher than the postoperative CAT level ($p = 0.050$). The postoperative TT level in Group 2 was higher than the preoperative and intraoperative levels ($p < 0.008$, $p < 0.008$, respectively). In Groups 1, 2, and 3, the intraoperative NT levels were significantly lower than the preoperative and postoperative NT levels ($p = 0.002$, $p = 0.001$, and $p = 0.001$, respectively). The intraoperative and postoperative TOS levels in Group 2 were higher than the preoperative TOS levels ($p = 0.001$). In Group 3, the intraoperative and postoperative MDA levels were higher than the preoperative MDA levels ($p = 0.001$) (Table 3).

In Group 2, there was a negative correlation between the preoperative TAS level and the postoperative intubation time ($p = 0.035$). As the preoperative TAS level decreased, the intubation time was prolonged. A positive correlation was noted between the postoperative NT level and the postoperative intubation time in Group 2 ($p = 0.005$). As the intubation period was prolonged, the postoperative NT level increased. In Group 3, there was a positive correlation between the postoperative CAT level and the postoperative intubation period ($p = 0.038$). As the intubation period was prolonged, the postoperative CAT level increased.

Discussion

This study investigated oxidative stress parameters related to different mechanical ventilation strategies in patients undergoing on-pump cardiac surgery. Mechanical ventilation (3–4 $mL.kg^{-1}$) without PEEP seems to be the most advantageous strategy. NT, which is an important antioxidant agent in humans, increased significantly in Groups 1, 2, and 3 in the postoperative period compared to the intraoperative period. However, only in group 2, TT level increased

Table 1 Demographic and clinical information.

			Group 1 (n = 20)	Group 2 (n = 20)	Group 3 (n = 20)	KW/χ^2/F	*p*-value
Age		Median (Q1–Q3)	63.50 (56.0–72.0)	58.00 (40.5–69.5)	52.00 (40.0–67.5)	3.736[x]	0.154
Sex	Female	n (%)	10 (50.0)	10 (50.0)	12 (60.0)	0.536[z]	0.765
BMI**		Mean ± SD	28.81 ± 3.43	28.67 ± 5.05	28.46 ± 5.01	0.030[y]	0.971
Smokers		n (%)	3 (15.0)	1 (5.0)	1 (5.0)	1.745[z]	0.418
Diabetes Mellitus	Yes	n (%)	10 (50.0)	4 (20.0)	6 (30.0)	4.200[z]	0.122
	No	n (%)	10 (50.0)	16 (80.0)	14 (70.0)		
Hypertension	Yes	n (%)	15 (75.0)	9 (45.0)	6 (30.0)	8.400[z]	**0.015***
	No	n (%)	5 (25.0)	11 (55.0)	14 (70.0)		

KW, Kruskal-Wallis H test; χ^2, chi-square test; F, Fisher's exact test; Anova Test Statistics; ** one-way ANOVA; α: 0.05; post-hoc: Tukey's HSD test; * the difference between groups is statistically significant; [x]Kruskal Wallis H test; [y] one-way ANOVA (F); [z] Chi-square/Exact (χ^2); BMI: body mass index.

Table 2 Surgery information.

		Group 1		Group 2		Group 3		KW/F/X2	*p*-value
		N	%	N	%	N	%		
Types of surgery	CABG	14	70.0	9	45.0	10	50.0	13,558[z]	0.845
	MVR	1	5.0	1	5.0	1	5.0		
	Aortic aneurysm	0	0.0	1	5.0	2	10.0		
	AVR	1	5.0	1	5.0	1	5.0		
	ASD	2	10.0	2	5.0	3	15.0		
	Atrial myxoma	1	5.0	0	0.0	0	0.0		
	CABG + MVR	0	0.0	2	10.0	0	0.0		
	AVR + MVR	0	0.0	2	10.0	2	10.0		
	TVR + MVR	1	5.0	1	5.0	0	0.0		
	VSD	0	0.0	1	5.0	1	5.0		
IO Fluid (mL)	*Median (Q1–Q3)*	675.00 (525.00–700.00)		550.00 (400.00–675.00)		500.00 (375.00–600.00)		4.450[x]	0.108
IO Urine**(mL)	*Mean ± SD*	1025.00 ± 498.00		1270.00 ± 505.00		1204.00 ± 704.00		0.964[y]	0.388
Left Ventricle E/F	*Median (Q1–Q3)*	55.00 (50.00–60.00)		57.50 (52.50–60.00)		57.50 (55.00–60.00)		1.037[x]	0.595
CPB Duration (min)	*Median (Q1–Q3)*	98.00 (72.00–117.50)		128.50 (99.00–162.00)		102.00 (79.00–121.50)		5.157[x]	0.076
Cross-clamp Time (min)	*Median (Q1–Q3)*	57.50 (41.00–95.00)		76.50 (57.00–124.00)		62.00 (43.00–95.00)		2.136[x]	0.344
Operation Time**	*Mean ± SD*	245.00 ± 54.90[b]		300.75 ± 66.34[a, c]		248.00 ± 65.18[b]		5.058[y]	**0.010***
Postoperative Intubation Time (min)	*Median (Q1–Q3)*	460.00 (360.00–705.00)		345.00 (255.00–697.50)		375.00 (315.00–570.00)		2.385[x]	0.303
ICU Stay (days)	*Median (Q1–Q3)*	3.00 (3.00–4.00)		3.00 (3.00–4.00)		3.00 (2.50–4.50)		0.135[x]	0.935

KW: Kruskal-Wallis H test; χ^2: chi-square test; Exact test; F:Anova Test Statistics; ** one-way ANOVA; α: 0.05; post-hoc: Tukey's HSD test; [x]Kruskal Wallis H test;Post-hoc: Dunn Sidak test; [y] one-way ANOVA (F); [z] Exact test (χ^2)* difference between groups is statistically significant; [a] the difference between this group and Group 1 is statistically significant; [b] the difference between this group and Group 2 is statistically significant; [c] the difference between this group and Group 3 is statistically significant; CABG: Coronary artery bypass graft; MVR: Mitral valve replacement; AVR: Aortic valve replacement; ASD: Atrial septal defect; TVR: Tricuspid valve replacement; VSD: Ventricular septal defect. IO: Intraoperative; E/F: Ejection/Fraction; CPB: Cardiopulmonary bypass; ICU: intensive care unit; Fluid: NaCl.0.9%.

in the postoperative period compared to the preoperative and intraoperative periods. In Groups 2 and 3, the postoperative TAS levels were higher than the intraoperative level. The postoperative MDA levels of Group 3 were significantly higher than the preoperative levels.

Mentese et al. compared the two groups according to the median aortic cross clamping (XC) time: Group 1 (XC time < 42 minutes) and Group 2 (XC time ≥ 42 minutes). TOS and oxidative stress index (OSI) values of all patients were higher 30 minutes after reperfusion than they had been preoperative, whereas the perioperative TAS values were similar to

Table 3 Comparison of the preoperative, intraoperative, and postoperative oxidative stress levels.

	Group 1 (n = 20) *Median (Q1–Q3)*	Group 2 (n = 20) *Median (Q1–Q3)*	Group 3 (n = 20) *Median (Q1–Q3)*	*p-value*
Preoperative GPX	0.011 (0.007–0.014)	0.014 (0.011–0.019)^{z}	0.01 (0.008–0.013)^{y}	**0.024***
Intraoperative GPX	0,010 (0.004–0.015)	0.012 (0.010–0.015)	0.014 (0.007–0.025)	0.260
Postoperative GPX	0,009 (0.005–0.013)	0.013 (0.012–0.015)^{z}	0.009 (0.006–0.011)^{y}	**0.017***
p	0.538	0.297	0.350	
Preoperative CAT	6.09 (2.68–10.56)^{c}	10.70 (5.21–20.77)	6.58 (2.64–11.79)	0.423
Intraoperative CAT	10.07 (3.66–15.07)	12.74 (9.22–19.57)^{c}	8.55 (4.12–14.89)	0.218
Postoperative CAT	13.76 (8.80–18.02)^{a}	5.77 (0.99–23.80)^{b}	10.38 (6.27–25.03)	0.085
p	**0.001***	**0.050***	0.522	
Preoperative TAS	3.07 (2.97–3.19)^{z}	3.37 (3.10–3.64)^{z}	4.32 (4.14–4.37)$^{x, y}$	**< 0.001***
Intraoperative TAS	3.01 (2.91–3.26)^{z}	3.26 (3.00–3.59)$^{c, z}$	4.32 (4.20–4.35)$^{c, x, y}$	**< 0.001***
Postoperative TAS	3.13 (3.05–3.26)$^{y, z}$	3.79 (3.59–4.16)$^{b, x, z}$	4.46 (4.334.67)$^{b, x, y}$	**< 0.001***
p	0.259	**0.001***	**0.019***	
Preoperative MDA	1.52 (1.31–1.86)^{z}	1.48 (1.27–1.92)	1.29 (1.08–1.41)$^{b, c, x}$	**0.043***
Intraoperative MDA	1.33 (1.17–2.89)^{z}	1.49 (1.40–2.67)^{z}	2.78 (2.45–3.08)$^{a, x, y}$	**0.003***
Postoperative MDA	1.64 (1.36–1.91)^{z}	1.43 (1.25–1.80)^{z}	3.45 (2.95–3.94)$^{a, x, y}$	**< 0.001***
p	0.522	0.949	**0.001***	
Preoperative TOS	19.65 (16.50–29.80)	17.20 (12.40–26.50)$^{b, c, z}$	41.30 (19.55–56.70)^{y}	**0.012***
Intraoperative TOS	28.00 (22.95–34.55)^{y}	86.50 (67.10–110.00)$^{a, x, z}$	51.10 (35.55–70.60)^{y}	**< 0.001***
Postoperative TOS	18.60 (17.65–25.85)$^{y, z}$	41.80 (34.20–53.10)$^{a, x}$	37.05 (33.95–42.65)^{x}	**< 0.001***
p	0.091	**0.001***	0.387	
Preoperative TT	318.28 (277.82–404.27)	334.70 (310.82–369.34)^{c}	337.69 (147.35–368.74)	0.797
Intraoperative TT	337.39 (290.96–392.48)^{z}	336.79 (312.61–388.15)$^{c, z}$	287.97 (271.70–323.36)$^{x, y}$	**0.017***
Postoperative TT	411.58 (355.15–440.40)	450.25 (358.29–475.33)$^{a, b}$	358.29 (302.90–452.34)	0.907
p	0.212	**0.008***	0.058	
Preoperative NT	239.58 (206.31–538.11)^{b}	237.98 (212.60–266.34)^{b}	258.66 (226.25–284.89)^{b}	0.477
Intraoperative NT	208.12 (175.28–221.02)$^{a, c}$	194.69 (168.67–214.95)$^{a, c}$	190.10 (165.37–219.42)$^{a, c}$	0.802
Postoperative NT	238.19 (217.82–258.34)^{b}	252.05 (213.24–287.87)^{b}	241.81 (223.80–284.25)^{b}	0.444
p	**0.002***	**0.001***	**0.001***	

Kruskal Wallis H Test; Friedman test; Post hoc: Bonferroni test; Dunn-Sidak test; α: 0.05; * the difference between measurements is statistically significant; ^{a} the difference between this measurement and the preoperative measurement is statistically significant; ^{b} the difference between this measurement and the intraoperative measurement is statistically significant; ^{c} the difference between this measurement and the postoperative measurement is statistically significant. ^{x} the difference between this group and Group 1 is statistically significant; ^{y} the difference between this group and Group 2 is statistically significant; ^{z} the difference between this group and Group 3 is statistically significant; GPx: glutathione peroxidase (u.mL^{-1}), MDA: malondialdehyde (nmol.mL^{-1}), CAT: catalase (u.mL^{-1}), TAS: total antioxidant status mmol.L^{-1}, TOS; total oxidant status μmol.L^{-1}, TT: total thiol (μmol.L^{-1}), NT: native thiol (μmol.L^{-1}).

the preoperative levels.[15] The cross-clamp time and the TOS levels were correlated at the 30th minute following reperfusion. Also, the TOS and OSI values of the group (aortic cross-clamping time $\geq$ 42 minutes) 30 minutes after reperfusion were higher than the preoperative values; no significant difference was found between the corresponding levels for the group (aortic cross-clamping time < 42 minutes).[15] Aortic cross-clamp time was correlated positively with an oxidative stress injury.[30] In the present study, although not statistically significant, the cross-clamp time and duration of CPB was longer, and postoperative intubation time was shorter in Group 2. In addition, the operation time was statistically longer in Group 2. However, in Group 2, the intraoperative and postoperative TOS levels were higher than the preoperative levels. In Groups 2 and 3, the postoperative TAS levels were higher than the intraoperative level. Hence, the TOS values in our study paralleled those of a previous study,[15] but TAS values did not. This difference may be due to the mechanical ventilation strategies in Groups 2 and 3. Another study revealed a significant decrease in the postoperative TAS levels in patients who underwent coronary artery bypass graft (CABG).[23] In the study by Luyten et al., the TAS level in plasma increased from 0.9 mM to 1.45 mM Trolox equivalent in 10 minutes of CPB, indicating a 60% increase in the plasma antioxidant capacity. Furthermore, the TAS values were significantly higher after protamine administration and in the 4th postoperative hour than the levels obtained preoperatively. No significant difference in the TAS was noted between the level at the 24th postoperative hour and the preoperative level.[24] In the present study, a significant difference was found between the intraoperative and postoperative periods in groups 2 and 3, with the TAS levels in both groups increasing postoperatively. Group 3 had the highest TAS value at the 24th postoperative hour.

In the same study,[24] the GPx level increased to an average of 20% in the first 10 minutes of CPB compared with the preoperative level, and it increased another 20% to reach 40% by the end of the cross-clamping. A significant difference was also noted between the preoperative period and the 24th postoperative hour, with the GPx levels increasing

postoperatively. In the present study, no significant in-group differences were found between the preoperative, intraoperative, and postoperative GPx levels in all three groups. However, Group 2 had the highest preoperative GPx level, with a significant difference compared to the preoperative level of Group 3. In a study to investigate the effects of 30 minutes of reperfusion after 60 minutes of severe global ischemia on the antioxidant enzymatic system in isolated perfused rat heart, ischemia caused a significant increase in GPx activity,[25] but in another study, in ischemic heart reperfusion, there was no increase in GPx levels both at the 15th and 45th minutes in CPB.[26]

Doğan et al. compared on-pump and off-pump groups. The MDA level of the on-pump group significantly increased during the intraoperative period (at the end of the surgical intervention) compared to the MDA levels preoperatively and postoperatively. The preoperative and intraoperative MDA levels were significantly higher in the on-pump group than the off-pump group. However, no significant differences were found in the postoperative period.[27] In groups 1 and 2 of our study, the changes from the preoperative period to the postoperative period were minimal and not significant. Conversely, the intraoperative and postoperative MDA levels of Group 3 were significantly higher than the preoperative levels. MDA showed an increase in Group 3 as an oxidative stress. This difference may be due to PEEP applied to Group 3. In another study, the lipid H_2O_2 levels increased significantly in the 1st and 4th hours following the start of CABG.[28]

The postoperative CAT activity in the on-pump group significantly decreased compared to its intraoperative activity. Regarding the GSH and CAT levels, the on-pump and off-pump groups had no significant differences during the preoperative, intraoperative, and postoperative periods.[27]

An intragroup analysis has indicated that the postoperative CAT level of Group 1 significantly increased compared to the preoperative level. Conforming to a previous study,[27] the postoperative CAT level of Group 2 in our study significantly decreased compared with its preoperative level. In a study in which a continuous mechanical ventilator strategy was used, the thiol and native thiol levels were significantly higher at 24 hours postoperatively than those of patients who were not ventilated.[29] In the present study, NT increased significantly in Groups 1, 2, and 3 in the postoperative period compared to the intraoperative period. However, only in Group 2, both NT and TT increased in the postoperative period compared to the preoperative and intraoperative periods. In Group 2 of our study, the time of intubation lengthened as the preoperative TAS value decreased; it also lengthened as the postoperative NT level increased. Furthermore, in Group 3, both the postoperative CAT level and the length of the intubation period increased.

The present study has some limitations including small sample size, single-center, and case diversity. The main limitation of this study is a clear imbalance between groups regarding preoperative characteristics of patients, which strongly impairs any conclusion and might have played a role in the findings of the present study.

In conclusion, the present study indicates that the maintenance of mechanical ventilation (tidal volume of 3 to 4 mL.kg^{-1} without PEEP) during CPB seems to be the most advantageous strategy in cardiac surgery. Additionally, prolonged postoperative intubation time was associated with both increased in NT and catalase levels. Future studies are still necessary to further investigate the role of mechanical ventilation during CPB and may focus on the effects of oxidative stress on the intubation time after surgery.

Conflicts of interest

The authors declare no conflicts of interest.

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