ORIGINAL ARTICLE

# **Hyperoxic oxidative stress during abdominal surgery: a randomized trial**

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### **Abstract**

*Purpose* The hypothesis of our study is that during anesthesia, administration of 80 % oxygen concentration increases oxidative stress more than 40 % oxygen.

*Methods* Forty ASA I-II patients were included in a randomized, single-blind study. Expiratory tidal volumes (ETV) were measured before induction and after extubation. After ventilation with  $0.8$  FiO<sub>2</sub> and intubation, mini-bronchoalveolar lavage (mini-BAL), arterial blood gas (ABG), and blood samples were taken. Patients were randomly assigned to receive 0.8 (group I) or 0.4 (group II)  $FiO<sub>2</sub>$ during management. Before extubation, mini-BAL, ABG, blood samples were taken. Pa $O<sub>2</sub>/FiO<sub>2</sub>$ , lactate, malondialdehyde (MDA), protein carbonyl (PCO), superoxide dismutase (SOD), total sulfhydryl (T-SH), non-protein sulfhydryl (NPSH), and protein sulfhydryl (PSH) were measured. In both groups, mean arterial pressure and heart rate values were recorded with 30-min intervals.

*Results* ETV values were higher in group II after extubation. PaO<sub>2</sub>/FiO<sub>2</sub> values were higher in group II after extubation compared to group I. In both groups, plasma PCO, SOD, and T-SH levels increased significantly before extubation, whereas the increase in MDA was not significant between groups. Plasma PCO, T-SH, and lactate levels were higher in group I, and plasma SOD, and PSH were higher in group I before extubation. In both groups, MDA,

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*Conclusions* We found that 80 % FiO<sub>2</sub> decreased ETV and  $PaO<sub>2</sub>/FiO<sub>2</sub>$  and increased lactate levels and oxidative stress more, inhibiting antioxidant response compared to 40 % FiO<sub>2</sub>.

**Keywords** Antioxidant response elements · Oxidative stress · Oxygen inhalation therapy · Postoperative pulmonary atelectasis

## **Introduction**

In recent years, studies have been published showing that high-concentration oxygen (>80 %) administration decreases the infection rate and has beneficial effects on wound healing in patients undergoing colorectal surgery [\[1](#page-8-0)[–3](#page-8-1)]. Nowadays, many anesthesiologists prefer to use 100 % oxygen during anesthesia induction, mainly for preoxygenation before endotracheal intubation and mainly in high-risk patients [[4\]](#page-8-2). Nonetheless, it is known that the use of high-fraction oxygen can lead to many problems. Especially during anesthesia, anesthetic agents, the use of neuromuscular blockers, position, surgical intervention, increase of oxygen gradient between the intra-alveolar oxygen pressure and mixed venous blood in the capillaries, and vanishing of the pulmonary hypoxic vasoconstriction leads to closing of the airways and alveolar collapse [\[5](#page-8-3)[–7](#page-8-4)]. Whatever the reason my be, atelectasis cause a reduction in gas exchange and lung volumes [[6,](#page-8-5) [7\]](#page-8-4). It is shown that in experimental animal lung studies, the inflammatory



response has been observed even in short periods of oxygen [[8\]](#page-8-6). Oxidative stress is one of the unwanted metabolic effects of high-fraction oxygen use [[5\]](#page-8-3). This effect arises from the mitochondria, where oxygen is the final acceptor for electrons in oxidative metabolism [\[9](#page-8-7)]. In this process, oxygen is reduced to water, and to different reactive oxygen species (ROS) such as superoxide radical, malondialdehyde (MDA), protein carboxyl groups (PCO), and hydrogen peroxide and hydroxyl radicals. Reactive oxygen species cause oxidative stress when the balance between the ROS and anti-oxidative (superoxide dismutase, total sulfhydryl-T-SH-, nonprotein sulfhydryl-NPSH-, protein sulfhydryl-PSH-) generation is disturbed [[9–](#page-8-7)[12\]](#page-8-8). Although oxidative stress is undetectable to the naked eye and it can be measured with mechanical-electronic devices and followed in routine blood biochemistry tests during anesthesia practice, it is a process that creates negative results on the organism. Under normal circumstances, the oxidative stress metabolism of the cells is controlled by cytochrome oxidase systems in the mitochondria. The incomplete reduction of oxygen causes ROS. ROS primarily causes a disturbance in the mitochondrion function by damaging the DNA. This pathology mostly affects brain and lung parenchyma [\[8](#page-8-6), [9](#page-8-7), [13](#page-8-9)].

The hypothesis of our study is that during anesthesia, administration of 80 % oxygen concentration increases oxidative stress more. To test this, we have evaluated the increase in the oxidative stress markers (MDA, PCO), the decrease in the antioxidant response (SOD, T-SH, NPSH, PSH), changes in arterial gas exchange, expiratory tidal volume, hemodynamics, and lactate levels in patients undergoing hemicolectomy.

# **Materials and methods**

## **Study design**

After ethical committee approval (I.U. Cerrahpasa Medical Faculty Ethical Committee no. 36048, date 18.12.2012) and patient consent, the prospective, randomized with allocation ratio of 1:1, single-blind, parallel arm clinical study was completed in the general surgery operation rooms of I.U. Cerrahpasa Medical School. The study was registered to the Clinical Trials of the US National Institutes of Health (Clinical trials number NCT01793454, Principal Investigator Guniz M. Koksal, Date of registration February 14, 2013).

# **Study population**

We assessed the eligibility of all ASA I-II patients, with no concomitant disease, weighing between 60 and 80 kg, aged between 20 and 60 years old, undergoing colon cancer surgery (left or right hemicolectomy). Patients were recruited from the general surgery clinic of our hospital. All patients included in the study were Turkish patients living in Istanbul, Turkey.

Patients undergoing total colectomy, with known diabetes mellitus, chronic obstructive pulmonary disease (hypoxic and hypercarbic), muscle-bone-related diseases, inflammatory bowel diseases (Crohn's disease, ulcerative colitis) diagnosis, and patients with malnutrition were not included in the study. During the study interventions, if any patient needed more than 1 U of erythrocyte suspension or vasopressor use, the intervention was discontinued. The need for more than 1 IU blood-bank erythrocyte suspensions or vasopressors is a sign of impairment of the perfusion. In patients with impaired perfusion, oxidative stress is influenced due to its effect on the inflammatory process. Hence, these patients were excluded from the study.

#### **Study interventions**

The patients were passively warmed until they were taken to the operation room. Mean arterial pressure (MAP), heart rate (HR), and expiratory tidal volumes (ETV) measured by "Wright Spirometer" were recorded prior to premedication. A 20-gauge cannula was inserted from the dorsum of the left hand and an infusion of 5 ml/kg/h balanced electrolyte solution (Isolyte-S, Baxter, Istanbul, Turkey) was started. Before using the Wright Spirometer for the study, during the preanesthetic assessment, all patients were informed of how to take breaths and to exhale into the spirometer [\[14](#page-8-10)]. Each patient had one trial experience. In the morning of the surgery, patients were taken to the preanesthetic room, and after a resting period of 15 min, the first measurements were done. The person doing the ETV measurements was an anesthesiologist who was unaware of the group of the patient.

Premedication was achieved with 2 mg midazolam IV (Dormicum, Roche). The patients were transferred to the operating room and as a standard procedure, three-lead ECG,  $SpO<sub>2</sub>$ , non-invasive arterial pressure, and skin temperature monitoring were performed (Datex Ohmeda S/5 Avance). Anesthesia was induced with 2 mg of midazolam, 1.5 mg/kg of propofol (Propofol %1 Fresenius), 0.01 mg/kg/h remifentanil infusion (Ultiva, GlaxoSmithKline), and patients were intubated with 0.6 mg/kg IV rocuronium (Esmeron, MSD). After the induction, radial artery and central venous catheterization from the right internal jugular vein were achieved. All patients in both groups received standard volume infusions. Isolyte S, which is a balanced crystalloid solution, was infused with a rate of 5 ml/kg/h. Each patient was given 500 ml of 6 % hydroxyethyl starch 130/0.4 in 0.9 % sodium chloride injection at 30 min following the anesthesia induction. Mean urine output was targeted as 0.5 ml/kg/h and central venous pressure under mechanical ventilation was targeted for 8–12 mmHg.

Patients in both groups were ventilated with  $0.8 \text{ FiO}_2$ in 6 l/min oxygen/air mixture during anesthesia induction. Following endotracheal intubation, before mechanical ventilation was started, patients were ventilated manually and as manual ventilation was stopped for a brief time, the first mini-BAL and plasma samples were taken (approximately 10 min after the anesthesia induction) through the intubation tube. Concurrently, arterial blood gas samples for the PaO<sub>2</sub>/FiO<sub>2</sub> ratio, lactate values, and blood samples for oxidative stress and antioxidant response measurements in the plasma were obtained. In both groups, mean arterial pressure (MAP) and heart rate (HR) values were recorded throughout the operation at 30-min intervals. Mini-BAL (blind BAL via Combi-Cath) sampling was performed by injecting 20 ml 0.9 % NaCl through a plugged tip-telescoping catheter (Combicath, Plastimed, France) inserted through the intubation tube and aspirating it back (mean,  $2-4$  ml)  $[15]$  $[15]$ .

After the first mini-BAL sample, patients were randomly assigned to either receive 0.8  $FiO<sub>2</sub>$  or 0.4  $FiO<sub>2</sub>$  during anesthesia management (0.8 FiO<sub>2</sub> in group I, 0.4 FiO<sub>2</sub> in group II). In addition, anesthesia was managed by 5 mg/ kg/h propofol and 0.05 µg/kg/h remifentanil infusions, in 4 l fresh gas flow of oxygen/air with fractioned rocuronium doses. In both groups, patients were ventilated with pressure-controlled mode to achieve tidal volumes between 6 and 8 ml/kg (ideal weight),  $6-8$  cmH<sub>2</sub>0 positive end-expiratory pressure (PEEP), 1:2 inspiratory to expiratory ratio and respiratory rate was adjusted to keep ETCO<sub>2</sub> levels between 35 and 40 mmHg. Prior to the second mini-BAL sampling, a recruitment maneuver was applied (vital capacity maneuver, inflation of the lungs up to  $40 \text{ cm}H<sub>2</sub>0$  and maintaining it for 15 s).

The second samples (mini BAL and plasma) were again taken at the end of the operation, before extubation (approximately 200 min after the anesthesia induction). MAP and HR values are recorded. All blood and mini-BAL samples were centrifuged, placed in Eppendorf tubes, and frozen at −80 °C. Plasma and mini-BAL samples were defrosted and studied at the same time.

For postoperative pain management, 100 mg tramadol IV (Contromal, Abdi İpekçi) and 2 g metamizole IV (Adepiron, Adeka) were given. The neuromuscular blockade was reversed with 2 mg/kg sugammadex (Bridion, MSD) and patients were extubated when spontaneous breathing was adequate. Following the extubation, they were taken to the post-anesthesia care unit. All patients received 3 l/min oxygen via facemask, and peripheral oxygen saturations were monitored. Patient-controlled analgesia was started with 2 mg bolus IV morphine without basal rate (Morfin HCl, OSEL) and with 10-min lock time to keep the visual analog scale (VAS) under 4. On 60th min of the extubation, ETV was measured with a "Wright Spirometer". ETV measurements were done in patients with Aldrete scores 9 or higher and VAS scores below 4.

# **Biochemical measurements**

#### *Assay of malondialdehyde (MDA)*

The rate of lipid peroxidation was measured with the Buege and Aust [[16\]](#page-8-12) procedure. MDA and its byproducts generate a colored product, and when reacted with thiobarbituric acid, this absorbs light at 535 nm maximally. This represents the color of generated by TBA reactive substances (TBARS).

#### *Assay of the superoxide dismutase activity (SOD)*

Superoxide dismutase (Cu–Zn SOD) activity in supernatant fractions was measured with the method of Sun and coworkers [[17\]](#page-8-13). This method uses the inhibition of nitroblue tetrazolium reduction; xanthine oxidase is used as a superoxide generator. One unit of SOD is defined as the amount of enzyme needed to show a 50 % dismutation of superoxide radical.

#### *Assay of protein carbonyl levels (PCO)*

PCO groups were determined using spectrophotometry, using the Reznick and Packer method [\[18](#page-8-14)]. These groups react with 2,4-dinitrophenylhydrazine (DNPH) and generate chromophoric dinitrophenylhydrazones. After DNPH reaction, proteins were precipitated with 20 % (w/v) trichloroacetic acid and washed three times with 4 ml of an ethanol/ethyl acetate mixture (1:1). A small spatula was used mechanically to disrupt the pellets in the washing solution, and it was re-pelleted by centrifugation at  $6000 \times g$  for 5 min. At the end, 6 M-guanidine-HCl solution was used to dissolve the protein precipitates, and the absorbance values were measured at 360 nm with the molar extinction coefficient of DNPH, *ε* = 22,000/M/cm. Protein contents of the supernatant fractions were determined by Bradford assay [\[19](#page-8-15)].

#### *Estimation of thiol groups*

Total sulfhydryl (T-SH), non-protein sulfhydryl (NPSH), and protein sulfhydryl (PSH) concentrations in serum and BAL were determined as redox-sensitive biomarkers, using 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) as described by Sedlak and Lindsay [[20\]](#page-9-0). Twenty-μl aliquots of the serum were mixed in 1.5-ml test tubes with 400  $\mu$ l of  $0.2$  M Tris buffer, pH 8.2, and 20  $\mu$ l of 0.01 M 5,5-dithiobis 2-nitrobenzoic acid (DTNB) for the determination T-SH groups. NPSH samples were assayed in the following way: 20 μl of plasma was mixed in 400 μl of 50 % TCA. The tubes were mixed intermittently for 10 min and centrifuged for 15 min at approximately  $3000 \times g$ . Supernatant fractions were assayed as T-SH. The absorbance values of the resulting samples were read at 412-nm wavelengths against reagent blank. The value of molar extinction coefficient of thiol (-SH) groups at wavelength 412 nm is approximately  $\varepsilon = 13,100/M/cm$ . The PSH groups were calculated by subtracting the NP-SH from T-SH [[19–](#page-8-15)[21\]](#page-9-1).

## **Randomization**

After patients were excluded according to eligibility screening by one of the authors (GM Koksal), the remaining patients were randomized. We generated the two comparison groups using simple randomization, and a computer random number generator with an equal allocation ratio. The biochemistry studies of oxidative stress and antioxidant response were performed by a researcher who was blinded to the group assignment.

#### **Study outcomes**

As the primary outcome, we have evaluated the effects of different inspired oxygen fractions on oxidative stress and antioxidant response in plasma and bronchoalveolar lavage after intubation and prior to extubation.

The secondary outcome was evaluating the effects of different inspired oxygen fractions on hemodynamics.

## **Statistical analysis**

All data are expressed as mean  $\pm$  SD with 95 % confidence intervals. The differences between demographic data, after induction, and before extubation values within groups were analyzed with paired t test, and the differences between two groups at two measurement points were analyzed with unpaired *t* test. NPSH and PSH data (non-homogenously distributed) were compared using the Mann–Whitney *U* test. *p* < 0.05 was considered significant. SPSS IBM, Utah 20.0 Statistical program (IBM, SPSS Statistics, Chicago, IL, USA).

# **Results**

# **Study population**

problems.

patients meeting the inclusion criteria were randomized into two groups  $(n = 20)$ . All randomized patients completed the trial (Fig. [1](#page-4-0)). No significant differences were found in demographic characteristics and perioperative hemodynamic values between groups (Table [1](#page-5-0)).

In both groups, hemodynamic values remained unchanged (Table [1\)](#page-5-0). All patients were extubated and were sent to the ward without complications.

In group I, ETV values were lower after extubation when compared with the values before intubation. The ETV values were higher in group II after extubation. ETV values after extubation were higher in group II compared to group I (Table [2;](#page-5-1) Fig. [2](#page-5-2)).

Lactate levels increased in both groups I and II before extubation compared to after intubation. Before extubation lactate values were higher in group I compared to group II (Table [2\)](#page-5-1).

In group I the  $PaO<sub>2</sub>/FiO<sub>2</sub>$  ratio decreased before extubation (Table [2\)](#page-5-1), conversely in group II this ratio increased before extubation. There was no difference in  $PaO<sub>2</sub>/FiO<sub>2</sub>$ ratios of two groups after intubation, but before extubation PaO<sub>2</sub>/FiO<sub>2</sub> ratios were higher in group II (Table [2\)](#page-5-1).

# **Biochemical markers of oxidative stress in plasma**

In both groups, plasma MDA, PCO, SOD, T-SH, and PSH levels increased before extubation, but the difference in MDA between groups was not significant, whereas the plasma PCO, T-SH levels were significantly higher in group I and SOD, and PSH levels were higher in group II before extubation. The NPSH levels in plasma decreased before extubation in both groups with no significant difference between groups (Table [3](#page-6-0)).

# **Biochemical markers of oxidative stress in mini‑BAL**

In both groups, BAL-MDA, PCO, SOD, T-SH, NPSH, and PSH levels increased before extubation compared to after intubation. In between-group comparison, MDA levels were significantly higher in group I, and PCO, T-SH, NPSH, and PSH levels were significantly higher in group II (Table [4](#page-6-1)).

All patients were extubated, and no patient was discharged to intensive care unit related to respiratory

# **Discussion**

In chronic diseases such as colorectal cancer, in high mortality situations like sepsis, and with inhalation anesthetics use, oxidative/anti-oxidative equilibrium shifts to the direction of oxidative stress  $[10-12, 15, 16, 22, 23]$  $[10-12, 15, 16, 22, 23]$  $[10-12, 15, 16, 22, 23]$  $[10-12, 15, 16, 22, 23]$  $[10-12, 15, 16, 22, 23]$  $[10-12, 15, 16, 22, 23]$  $[10-12, 15, 16, 22, 23]$  $[10-12, 15, 16, 22, 23]$  $[10-12, 15, 16, 22, 23]$  $[10-12, 15, 16, 22, 23]$ . We have

A total 168 patients undergoing colorectal surgery between February 1, 2013 and May 31, 2013 were screened and 40

## <span id="page-4-0"></span>**Fig. 1** Flow diagram



used propofol instead of inhalation anesthetics for maintenance not to further increase oxidative stress [\[10](#page-8-16)[–12](#page-8-8)]. In both groups, the patients without any preexisting lung pathology were ventilated with "lung protective mechanical ventilation" strategy using the same parameters. Patients in both groups were operated with the same surgical techniques for the same type of surgical pathologies. All these parameters were kept constant. The only variable parameter was the oxygen concentration (80 vs. 40 %). Thus, we have attributed the changes in the clinical condition and biochemical markers (positive and negative) to the oxygen concentration variable.

"Intraoperative lung protective mechanical ventilation" strategy prevents the full closing of the alveoli at the end of the expiration by the administration of "positive end expiratory pressure and recruitment maneuvers", and opens the collapsed atelectasis. It assures keeping the lung volumes open under mechanical ventilation, which will ensure the gas exchange. This is achieved by restoring the functional residual capacity [[24,](#page-9-4) [25\]](#page-9-5). In our opinion, it is possible that in the patients that we have administered 40 % oxygen, atelectasia that was present before induction in room oxygen (21 %), were opened by "lung protective mechanical ventilation" and the formation of new atelectasia was prevented, improving oxygenation and ETV values. However,

<span id="page-5-0"></span>**Table 1** Demographic data  $(mean \pm SD)$ 

	Group I $n = 20 (80 \%)$	Group II $n = 20 (40 \%)$	$\boldsymbol{p}$
Age (year)	$48.4 \pm 15.4$	$52.5 \pm 15.3$	0.638
Sex (F/M)	13/7	15/5	0.801
Weight (kg)	$72.5 \pm 13.6$	$71.2 \pm 14.1$	0.741
Height (cm)	$163.7 \pm 7.5$	$162.3 \pm 7.8$	0.923
ASA (I/II)	I(12)/II(8)	I(11)/II(9)	0.561
Operation duration (min)	$201.6 \pm 16.6$	$210.1 \pm 12.5$	0.140
Mean arterial pressure (mmHg)	$85.0 \pm 13.0$	$89.0 \pm 10.0$	0.680
Heart rate (beat/min)	$90 \pm 14.0$	$93.0 \pm 12.0$	0.441
Postext mean arterial press (60 min) (mmHg)	$88.3 \pm 11.2$	$87.8 \pm 12.5$	0.778
Postext heart rate (60 min) (beat/min)	$91.7 \pm 10.4$	$89.6 \pm 13.2$	0.354
Postoperation VAS (60 min)	$3.1 \pm 0.6$	$3.0 \pm 0.8$	0.315
Aldrete scores	$9.8 \pm 1.9$	$9.84 \pm 1.7$	0.496

*Postext* postextubation

The differences between after induction and before extubation demographic data within groups were analyzed with paired *t* test

<span id="page-5-1"></span>**Table** 2 PaO<sub>2</sub>/FiO<sub>2</sub> (*P/F*) ratio, lactate (mmol/l) levels, and ETV (ml) in all groups (mean  $\pm$  SD)

	Group I $n = 20 (80 \%)$	Group II $n = 20 (40 \%)$	$p$ (between groups)	$p$ (within group I)	$p$ (within group II)
$P/F_1$	$215.5 \pm 17.6$	$209.5 \pm 24.3$	0.66		-
$P/F_{2}$	$184.9 \pm 14.1$	$238.7 \pm 16.8$	0.0004	0.038	< 0.0001
Lactate <sub>1</sub>	$1.33 \pm 0.1$	$1.39 \pm 0.1$	0.15		-
Lactate,	$2.41 \pm 0.2$	$1.66 \pm 0.1$	0.0039	<0.0001	0.0066
$ETV_1$	$551.0 \pm 26.3$	$544.5 \pm 26.4$	0.24		
$ETV_{2}$	$419.3 \pm 21.0$	$589.5 \pm 27.2$	< 0.0001	< 0.0001	0.0022

The differences between after intubation and before extubation *P/F* and lactate data within groups were analyzed with paired *t* test and the differences between two groups at two measurement points were analyzed with unpaired *t* test

 $P/F_1$  *P/F* ratio after intubation,  $P/F_2$  *P/F* ratio before extubation, *Lactate<sub>1</sub>* lactate levels after intubation, *Lactate<sub>2</sub>* lactate levels before extubation, *ETV1* expiratory tidal volumes before induction, *ETV2* expiratory tidal volumes after extubation, *p* within groups first measurement compared with second measurement



**Expiratory Tidal Volumes (ETV) in all groups (mL)** 

<span id="page-5-2"></span>**Fig. 2** Expiratory tidal volumes (ETV) in all groups (ml). \*\*\* $p = 0.0001$ , \*\* $p = 0.00022$  within groups first measurement compared with second measurement, †††*p* = 0.0001 compared between groups. The differences between before induction and after extubation expiratory tidal volume data within groups were analyzed with paired *t* test and the differences between two groups at two measurement points were analyzed with unpaired *t* test

in the group where 80 % oxygen was administered, we observed that "lung protective mechanical ventilation" did not prevent the fast-formed atelectasis due to hyperoxia and consequently  $PaO<sub>2</sub>/FiO<sub>2</sub>$  ratio and expiratory tidal volumes (ETV) decreased. We did not perform a recruitment maneuver after the first mini-BAL sampling taken following the induction. Because we considered that applying high pressures before the start the surgery where we were not able to deliver adequate fluid replacement, can impair the hemodynamics of the patient resulting with hypotension. We may conclude that "lung protective mechanical ventilation and recruitment maneuvers" strategy does not accomplish its anticipated effect under 80 % oxygen concentration. While no significant difference was observed between groups after intubation, the  $PaO<sub>2</sub>/FiO<sub>2</sub>$  ratios decreased in group I significantly. This also suggests that high FiO<sub>2</sub> may lead to atelectasis. Hedenstierna et al.  $[26]$  $[26]$ have determined that the recruitment maneuvers done with

	Group I $n = 20 (80 \%)$	Group II $n = 20 (40 \%)$	$p$ (between groups)	$p$ (within group I)	$p$ (within group II)
MDA <sub>1</sub>	$5.0 \pm 1.4$	$5.6 \pm 4.0$	0.546		
MDA <sub>2</sub>	$8.1 \pm 2.9$	$8.1 \pm 3.5$	0.224	0.039	0.04
PCO <sub>1</sub>	$5.0 \pm 1.2$	$5.1 \pm 1.4$	0.909	-	-
PCO <sub>2</sub>	$7.5 \pm 1.5$	$5.8 \pm 2.87$	0.035	0.002	0.025
$SOD_1$	$0.9 \pm 0.3$	$1.2 \pm 0.8$	0.127	-	-
SOD <sub>2</sub>	$1.4 \pm 0.3$	$3.6 \pm 1.1$	< 0.0001	< 0.0001	< 0.0001
$T-SH_1$	$1.0 \pm 0.5$	$1.0 \pm 0.3$	0.11		
$T-SH2$	$2.4 \pm 0.3$	$1.3 \pm 0.5$	0.003	0.0015	0.035
$NPSH_1$	$5.9 \pm 0.4$	$5.6 \pm 3.3$	0.18		$\overline{\phantom{0}}$
NPSH <sub>2</sub>	$2.7 \pm 2.8$	$2.56 \pm 0.5$	0.26	< 0.0001	< 0.0001
$PSH_1$	$0.9 \pm 0.4$	$0.5 \pm 0.3$	0.164	$\overline{\phantom{0}}$	-
PSH <sub>2</sub>	$2.6 \pm 1.2$	$3.2 \pm 1.4$	0.029	0.007	< 0.0001

<span id="page-6-0"></span>**Table 3** Plasma oxidant and anti-oxidant values (nmol/mg pr) (mean  $\pm$  SD)

The differences between after induction and before extubation MDA, PCO, SOD, T-SH data within groups were analyzed with paired *t* test and the differences between two groups at two measurement points were analyzed with unpaired *t* test. NPSH and PSH data (non-homogenously distributed) were compared using the Mann–Whitney *U* test

*MDA1* malondialdehyde after intubation, *MDA2* malondialdehyde before extubation, *PCO1* protein carbonyl groups after intubation, *PCO2* protein carbonyl groups before extubation, *SOD<sub>1</sub>* superoxide dismutase activity after intubation, *SOD<sub>2</sub>* superoxide dismutase activity before extubation, *T-SH<sub>1</sub>* total thiol groups after intubation, *T-SH<sub>2</sub>* total thiol groups before extubation, *NPSH<sub>1</sub>* non-protein sulfhydryl groups after intubation, *NPSH2* non-protein sulfhydryl groups before extubation, *PSH1* protein sulfhydryl groups after intubation, *PSH2* protein sulfhydryl groups before extubation, *p* within groups; first measurement compared with second measurement

<span id="page-6-1"></span>**Table 4** Bronchoalveolar lavage oxidant and anti-oxidant values (nmol/mg pr) (mean  $\pm$  SD)

	Group I $n = 20 (80 \%)$	Group II $n = 20 (40 \%)$	$p$ (between groups)	$p$ (within group I)	$p$ (within group II)
MDA <sub>1</sub>	$4.2 \pm 2.4$	$3.8 \pm 1.2$	0.744		
MDA <sub>2</sub>	$12.6 \pm 2.9$	$7.7 \pm 3.1$	0.001	< 0.0001	< 0.0001
PCO <sub>1</sub>	$2.9 \pm 1.4$	$3.0 \pm 2.2$	0.12		-
PCO <sub>2</sub>	$4.5 \pm 1.8$	$10.1 \pm 3.2$	0.001	< 0.0001	< 0.0001
SOD <sub>1</sub>	$11.7 \pm 2.0$	$10.8 \pm 2.2$	>0.05	-	$\overline{\phantom{0}}$
SOD <sub>2</sub>	$13,4 \pm 2.3$	$13.7 \pm 2.2$	0.001	0.04	0.02
$T-SH_1$	$4.1 \pm 2.2$	$4.4 \pm 2.6$	0.454		-
$T-SH_2$	$7.9 \pm 3.9$	$14.5 \pm 8.6$	0.001	0.004	< 0.0001
$NPSH_1$	$0.5 \pm 0.5$	$0.6 \pm 0.5$	0.210	-	$\overline{\phantom{0}}$
NPSH <sub>2</sub>	$1.2 \pm 0.78$	$2.2 \pm 1.0$	0.001	0.03	< 0.0001
$PSH_1$	$5.5 \pm 3.8$	$4.3 \pm 2.8$	0.190		
PSH <sub>2</sub>	$6.7 \pm 2.4$	$11.8 \pm 8.4$	< 0.0001	0.028	< 0.0001

The differences between after induction and before extubation MDA, PCO, SOD, T-SH data within groups were analyzed with paired *t* test and the differences between two groups at two measurement points were analyzed with unpaired *t* test. NPSH and PSH data (non-homogenously distributed) were compared using the Mann–Whitney *U* test

*MDA<sub>1</sub>* malondialdehyde after intubation, *MDA*<sub>2</sub> malondialdehyde before extubation, *PCO<sub>1</sub>* protein carbonyl groups after intubation, *PCO*<sub>2</sub> protein carbonyl groups before extubation, *SOD<sub>1</sub>* superoxide dismutase activity after intubation, *SOD<sub>2</sub>* superoxide dismutase activity before extubation, *T-SH1* total thiol groups after intubation, *T-SH2* total thiol groups before extubation, *NPSH1* non-protein sulfhydryl groups after intubation, *NPSH2* non-protein sulfhydryl groups before extubation, *PSH1* protein sulfhydryl groups after intubation, *PSH2* protein sulfhydryl groups before extubation, *p* within group; first measurement compared with second measurement

high concentrations of oxygen during anesthesia were not able to prevent atelectasis in the postoperative period. Our findings support the findings of Hedenstierna et al. [[26\]](#page-9-6) who suggest that PEEP and recruitment maneuvers must be administered with lower oxygen concentrations. Edmark

et al. [[4\]](#page-8-2) grouped their patients into three and ventilated them with 100, 80, and 60 % oxygen during anesthesia induction for 5 min. No significant difference was observed in terms of  $PaO<sub>2</sub>$  and  $PaCO<sub>2</sub>$  levels. Although forced expiratory volumes were similar in all groups, computerized tomography scans showed an increase in atelectatic areas in patients ventilated with 100 % oxygen. As we did not study with FiO<sub>2</sub> levels of 25, 30, 35, and 45 % or values lower than 80 %, we do not know if the same effect would also be observed at those levels.

Martin et al. [[27\]](#page-9-7) showed that hyperoxia causes coronary vasoconstriction, increases peripheral vascular resistance, and decreases cardiac output. These results may cause hemodynamic changes. In our study, no significant difference was observed between groups, in contrast to Martin et al. [\[9](#page-8-7)].

Lactate levels were higher in group I (FiO<sub>2</sub> 80 %), resulting in a significant difference. Patients in both groups were protected from hypothermia during the intraoperative period by forced-air heaters. There was no difference between the fluid balances of the two groups. Lactate is one of the best indicators of perfusion. However, plasma lactate levels are also affected by the lactate clearance in the liver. Our patients did not have any liver function failure that could impair the lactate clearance. Habre et al. [[5\]](#page-8-3) noted that the increases in  $FiO<sub>2</sub>$  and oxygen transport capacity do not improve the oxygenation in the microcirculation. In contrast to that, an optimal cardiac output and a moderate hypercapnia increase tissue oxygenation more. While perioperative hyperoxia causes vasoconstriction in the systemic arteries, it also impairs coronary circulation. The increase in the systemic vascular resistance is directly proportional to an increase in afterload and a decrease in preload. Also, hyperoxia in the presence of anemia induces vasoconstriction and reduces the blood flow in the microcirculation [\[5](#page-8-3)]. Although we are not able to totally explain this lactate increase with the impairment of the perfusion, this issue shows that larger, prospective, clinical, randomized studies are needed.

Machado et al. [[8\]](#page-8-6) applied short-term oxygen therapy to New Zealand rabbits and compared it with rabbits in room air (20 and 75 min). They showed an inflammatory response with neutrophil and eosinophil predominance. However, our literature search revealed no study evaluating the oxidant and antioxidant levels in plasma and BAL samples during anesthesia. We also aimed to display the changes in lung parenchyma by evaluating mini-BAL samples besides peripheral blood samples, as the lung parenchyma is exposed to oxygen directly. As a study done on humans, we were not able to achieve a histopathological evaluation of the lungs and we were not able to find any study done on human subjects. In our study, we have evaluated the peripheral blood, and mini-BAL samples taken from the lungs simultaneously to reveal the increase in the oxidative stress and the antioxidant response the body gives to this increase. Failure inadequate antioxidant response is harmful to organism. An imbalance between the production and breakdown of ROS leas to oxidative stress and

potential harm [[9–](#page-8-7)[11,](#page-8-17) [28](#page-9-8), [29\]](#page-9-9). In peripheral and mini-BAL samples, we have measured MDA and PCO groups as oxidative stress markers. On this account, MDA and PCO show lipid and protein function loss on the cell membrane, which is a sign of destruction of cell integrity [[13,](#page-8-9) [30,](#page-9-10) [31](#page-9-11)]. Hence, we conclude that the increase in the oxidative markers can be observed both in the systemic and local circulation at the same time. Ranges of the markers that we used to track the oxidative stress and antioxidant response in the plasma and BAL samples are not wide. The normal values of the markers in the plasma and blood are not known. There must be some unknown mechanism or characteristics of the markers.

When we evaluate these results, we suggest that oxygen administration of 40 and 80 % increases the oxidative stress. In the BAL measurements, the increase in the MDA levels was lower in the group that 40 % oxygen was administered compared to the 80 % group but increase in PCO levels were higher. The reason for this may be due to the differences in the measurement techniques of the PCO, differences in their synthesis in BAL or plasma, or unknown characteristics of the PCO. Larger, clinical, prospective, randomized biochemical studies are needed on this subject.

We have measured the T-SH, NPSH, PSH, and SOD, which all have protective effects on free radicals and show antioxidant response. Especially SOD has been shown to prevent hyperoxia-induced acute lung injury by disturbing the deterioration in the alveoli in a study done on mice [\[13](#page-8-9)]. The SOD levels in mini-BAL and plasma samples measured before extubation were found to be increased. This increase is a sign of antioxidant response to oxidative stress. A healthy organism aims to maintain a balance between oxidative stress and antioxidant response. However, the decreases in  $PaO<sub>2</sub>/FiO<sub>2</sub>$  ratio and ETV levels give rise to the thought that antioxidant response is not enough in maintaining the balance. The dominancy of the oxidative stress in the group where 80 % oxygen was administered has resulted with closing of the alveoli and impairment in gas exchange.

Our study, for the first time on human subjects, shows that under anesthesia, a high concentration of oxygen alone increases oxidative stress independent from the duration of exposure. The decrease in antioxidant response also supports this finding.

When we evaluate the antioxidant response marker levels we have obtained, plasma and BAL levels increased in general. Only plasma NPSH levels decreased in both groups. It was also observed that NPSH levels in BAL samples increased less (especially in group I where 80 % oxygen was administered) compared to other markers in the BAL samples. The cause for this finding may be related to an increase in NPSH use in the plasma or a decrease in its synthesis. However, the increase in NPSH levels is

not even with the decrease in plasma levels. We believe that application of high-concentration oxygen directly to the lungs induced a faster and a more effective antioxidant response similar to oxidative stress. To verify this hypothesis, a larger study is needed with longer anesthesia durations where NPSH levels are observed.

BAL T-SH and PSH levels increased significantly in the group where 40 % oxygen was administered. Although SOD levels appear to be similar in both groups before extubation, the increase is higher in group II where 40 % oxygen was administered. While the antioxidant response markers in the plasma also increased, they were not as evident as the increase observed in the BAL samples. This suggests that the antioxidant response in the group where 40 % oxygen was administered was superior and the local effect was more distinct. This can be explained by the fact that the response starts following the occurrence of the oxidative stress. In conclusion, it can be stated that although administration of 40 % oxygen does not decrease oxidative stress compared to 80 % oxygen administration, it stimulates antioxidant response more and the mechanisms that balance the oxidative stress and antioxidant response. We believe novel biochemical studies are needed to show this mechanism.

However, changes in the group I (FiO<sub>2</sub> 80 %) were more marked and were accompanied by an increase in lactate levels, and a decrease in  $PaO<sub>2</sub>/FiO<sub>2</sub>$  ratio and ETV. This finding may be another reason besides anesthesia duration, in not observing hemodynamic changes in between groups and in group comparison.

As a first limitation of our study, the person who applied the mechanical ventilation and did the measurements was not blinded, and only the person who worked on the biochemical parameters was blinded and this could create an investigator bias. The lack of radiological verification of atelectasis is our second limitation. We believe the reason for the absence of difference is due to the short duration of surgery and anesthesia. This is the third limitation.

# **Conclusions**

In our study, where we used two different  $FiO<sub>2</sub>$  levels (40) vs. 80 %), we found that 80 %  $FiO_2$  decreased tidal volumes,  $PaO<sub>2</sub>/FiO<sub>2</sub>$  ratio, and increased lactate levels and oxidative stress more, inhibiting antioxidant response compared to 40  $%$  FiO<sub>2</sub>. More clinical studies are needed to further evaluate the effects of high oxygen fraction.

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#### **Compliance with ethical standards**

**Conflict of interest** The author(s) declare that they have no competing interests.

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