



Immunological (NK-cells, CD3, CD4 and CD8) and Non-Immunological (Lung Hyperinflation and Hyperventilation) and Hormonal; (Hydrocortisone) Effects of Large vs Small Tidal Volume Ventilation as a Lung Protective Strategy during General Anaesthesia

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Abstract

General anesthesia and surgical stress are known to influence patients' cellular immunity. Researches suggests that higher tidal volumes can injure healthy lungs, stimulate the release of inflammatory chemicals and predispose animals to organ damage through ventilator-induced lung injury.

The lung can be injured by positive pressure ventilation. Mechanical stretch triggers a pro-inflammatory response within the first 2 hours in healthy animal models. The benefit of Lung Protective Ventilation (LPV) with low Tidal Volumes (V_T), usually 6 mL/kg Predicted Body Weight (PBW), has been strongly beneficial for patients with acute lung injury and Acute Respiratory Distress Syndrome (ALI/ARDS). There is a concern of the potential injurious role of ventilatory over-distention in patients without lung injury.

Objectives and aim: To evaluate Immunological (NK-cells, CD3, CD4, and CD8) and non-immunological (lung hyperinflation and hyperventilation) and hormonal; (hydrocortisone) effects of small vs large tidal volume ventilation as a lung protective strategy during general anaesthesia.

Patients and methods: After approval of local ethical committee, patients written consent and sample size calculation, 60 ASA I and II physical status patients scheduled for plastic surgery were classified into two equal groups, each group had 30 patients (large TV group), LTVG and (small TV

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group), STVG. We compared patients receiving tidal volumes 12 mL/kg PBW or more with those that received 6 mL/kg PBW or less. All patients had large bore I.V. line (16-18 G). Material and method; ABGs were done three times; before induction, two hours after induction and in PICU.

Plain chest – x-rays were done three times; one after 2h of induction, one in recovery room and the third after six hours to diagnose hyperinflation and air trapping. All patients ≥ 20 years old who underwent elective plastic surgery of ≥ 3 hours were included in this study. The exclusion criteria were; patients with immune system or blood disease. Patients had fever, infection or received immunoregulatory (corticosteroids and azathioprine and bleomycin) drugs were excluded.

Results: There was no significant difference of patients' ages and operation time. There was a significant difference regarding sex, height and obesity, as most of LTV group were obese, short and females. Most of STV group were males, non-obese and tall. Three patients (10 %) in the LTV group received blood transfusion while only one case (3.3 %) in STV group needed. Blood hydrocortisone; serum level was the same just before induction, increased significantly after induction in both groups. After 2 hours, its significant increase continued in LTVG until the end of the procedure while its level started to decrease towards the end of operation in STVG. CD3, CD4 - T lymphocytes and CD4/CD8 ratio increased significantly after induction of anesthesia, 2h and at the end of the procedures especially at LTV group. NK cells decreased significantly in LTV group compared to STV group as it started after 2 hours of induction until the end of the procedure. Nineteen patients in LTV group and only 2 patients in STV group showed hyperinflation of the lungs after 2 h of induction. After 6 hour, 15 patients of LTVG showed hyperinflated lung as four patients recovered radiologically and no patients in small TV group showed hyperinflation. All LTVG patients were hyperventilated with PCO₂ (22-27 mm Hg \pm 1.4) and mild respiratory alkalosis (PH =7.29-7.31 \pm 0.5).

Conclusion: Small tidal volume ventilation is better tolerated by patients under general anaesthesia as large TV ventilation carries the risks of immune system dysfunction, stimulation of stress hormone release, hyperinflation and hyperventilation.

Introduction

There is a growing concern of the potential injurious role of ventilatory over-distention in patients without lung injury. Researches suggests that higher tidal volumes can injure healthy lungs, stimulate the release of inflammatory chemicals and predispose lungs to organ damage through so-called ventilator-induced lung injury. [1] the use of small tidal volumes (V_T) 6 mL/kg predicted body weight (PBW) has been recommended in healthy patients by some researches [2]. Others; explored the incidence and risk factors for receiving large tidal volumes ($V_T > 12$ mL/kg PBW) [3]. General anesthesia and surgical stress are known to influence patients' cellular immunity [4]. The lung can be injured by positive pressure ventilation. Mechanical stretch triggers a proinflammatory response within the first 2 hours in healthy animal models [1,4]. The benefit of lung protective ventilation (LPV) with low tidal volumes (VT), usually 6 mL/kg predicted body weight (PBW), has been strongly evidenced for patients with acute lung injury and acute respiratory

distress syndrome (ALI/ARDS) [2]. LPV strategies, designed to limit end-inspiratory volumes and pressures, were associated with reduced inflammatory markers in bronchoalveolar lavage fluid and blood and improved clinical outcomes [5]. In patients without evidence of existing lung injury, the significance of ventilator-induced lung injury is controversial. Clinical studies favoring a LPV regimen in non-ALI patients suggest a decreased inflammatory or pro-coagulation mediators with LPV strategies compared to conventional ventilation, and some have found improvement in clinical outcomes after thoracic or esophageal surgery [6]. Different manoeuvres as PEEP, oxygen fractions and concerns of potential effects of LPV (i.e. Atelectasis, Hypercapnia, etc.) have prevented from reaching widespread application [7]. Guidelines for intraoperative ventilation recommended a threshold for healthy patients has to set at $V_T < 10$ mL/kg PBW. Authors hypothesized that $V_T > 10$ mL/kg PBW is still often applied in routine intraoperative ventilatory set up [8]. They expected $a < 10\%$ of patients receiving unintentional large tidal volumes because of reduced height or obesity-related height/weight disproportion [3]. Large TV may affect patients' cellular immunity through several mechanisms such as hydrocortisone release, inhibition of NK cell and changes of T lymphocyte subpopulations. In the present study, we compared the effect of large vs small TV ventilation on human cellular immunity in patients undergoing plastic surgery through investigation of blood hydrocortisone, peripheral nature killer (NK) cells and T lymphocyte subpopulations. CD4 cells are the "generals" of the human immune system. They send signals to activate body's immune response when they detect viruses or bacteria. These cells fights off infections. So, it is important to keep their numbers in the normal ranges. A normal CD4 count can range from 500 cells/mm³ to 1,000 cells/mm³. Starting treatment when your CD4 count falls to 350 cells/mm³ or below. CD4 count can vary as much as 50-100 cells/mm³ from one day to the next. So, CD4 percentage is a more accurate measurement of immune function.

A CD4 $>29\%$ usually means that immune system is functioning normally (i.e., your CD4 count is roughly >500 cells/mm³).

A CD4 percentage of 14%-28% typically means CD4 count is in the range of 200-500 cells/mm³.

When CD4 count is < 200 cells/mm³, CD4 percentage is likely to be below 14%. C4, it is a potent bronchoconstrictor.

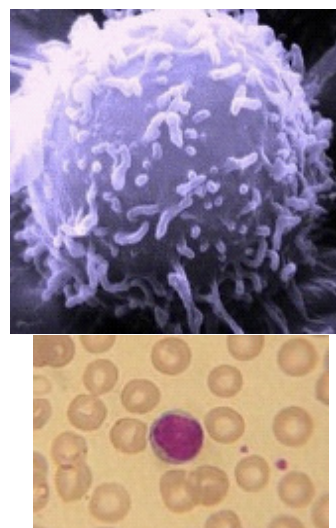


Figure 1: Human lymphocyte by EM.

The major subgroups of lymphocytes, namely T, B, and Natural Killer (NK) cells, can be distinguished by surface markers such as CD3, CD4 and CD8 [9].

Cluster of differentiation (CD)

B-lymphocytes are CD45+CD19+ and T lymphocytes are CD45+CD3+CD19. T lymphocytes can be further subdivided into T Helper (TH) cells (CD45+CD3+CD4+), cytotoxic T cells (CD45+CD3+CD8+), and activated T lymphocytes (CD45+CD3+CD25+). CD4 and CD8 serve in antigen recognition NK cells are activated in response to a family of cytokines called Interferons typical reference ranges of the major lymphocyte subpopulations in the blood of adults (according to Cell type surface molecule percentage of total lymphocyte population.

T cells CD3 69-93, T Helper cells CD4 35-72, Cytotoxic T cells CD8 7-33, activated T cells CD4 14-43, Activated T cells CD3 1-7, CD3, NK cells 3-31 [10].

When studying lymphocyte subpopulations, only lymphocytes in peripheral blood are sampled. The whole blood volume contains about 1010 lymphocytes, but these represent only about 2 % of the total lymphocyte pool in the human body, because most of the cells are in the lymphatic organs (Stress, Physical Exertion, Age, Ethnicity, Circadian Rhythms, and Medication) can affect the distribution of lymphocytes between blood and lymphatic tissue, as well as the pattern of lymphocyte subpopulations. Some disease states can influence the subpopulation patterns. Studies concerning the effect of metals on lymphocyte subpopulations have been carried out [11].

Serum Insulin-like Growth Factor (IGF)-I level is Growth Hormone (GH) dependent and reflects GH secretion (110- 115 ng/ml) is going to be of interest in determining the risk of many disorders such as ALI or ARDS. It is affected by age, gender, ethnicity, medications, chronic illness, or assay methodologies [12]. Alpha-2-Macroglobulin; A2M. Is a glycoprotein that consists of 36 exons. The protein is found in serum concentrations of 2-4 mg/mL. It consists of two identical subunits, which themselves are made up of two peptide chains covalently linked by disulfide bonds. Alpha-2M is one of the acute phase proteins. Plasma levels of Alpha-2M are reduced in patients with sepsis and ALI [13].

Patient and methods

After approval of local ethical committee, patient's written consent; sixty patients ASA I and II physical status nonsmokers; scheduled for plastic surgery (mamoplasty and abdominoplasty) were classified into two equal groups, each group had 30 patients (large TV group), LTVG and (small TV group), STVG. Sample size done according to the study done by Wrigge H. et al that showed: Patients equal to 30 patients in each group deemed sufficient for being the distribution of the sample means is fairly normally distributed. Therefore, the more samples one takes, the more the graphed results are satisfactory. We compared patients receiving tidal volumes of 12 mL/kg PBW or more with those that received 6 mL/kg PBW or less. At least 3 h duration was chosen to exclude short procedures, as mechanical ventilation might be too short to affect immune system or lungs. All data have been collected from clinical documentation sheet. Patient characteristics (age, gender, height, weight) intraoperative management (ventilatory settings, blood gas analysis, intravenous fluids and need of blood products), and postoperative course (need of Postoperative Mechanical Ventilation (POMV) and ICU admission, duration of mechanical ventilation, ICU and hospital length of stay, in-hospital mortality) were recorded. Pa-

tients' predicted body weight was calculated: Males: PBW (kg) = 50 + 0.91 × (height (cm)-152.4); Females: PBW (kg) = 45.5 + 0.91 × (height (cm) - 152.4). Recorded tidal volumes (V_T), in mL per kg PBW were calculated. Patients with median values of intraoperative VT 6 mL/kg PBW or less and, TV 12 mL/kg PBW or more were selected for comparison. Demographics, intraoperative management and outcomes from the two VT groups were compared to detect differences that may be implicated in the use of V_T 12 mL/kg PBW or more vs TV of 6ml/ kg PBW or less. Chest x-ray done 3 times; after 2 hours from induction, postoperatively in PACU and one more after 6 hours postoperatively.

All patients had large bored I.V. line (16-18 G) after LA infiltration to manage fluid balance and anaesthetic complications . ABGs were done three times; before induction ,after 2 hours of induction and at the end of procedure . Plain chest – x-rays were done 3 times; one after 2h of induction and one in recovery room to diagnose hyperinflation and air trapping one more after 6 hours postoperatively. All patients 20 - 50 years old scheduled for elective plastic surgery of ≥3 hours were included in this study. The exclusion criteria; were; patients with immune system or blood disease, smoker, asthmatics, patients with fever, infection or had received immunoregulatory (corticosteroids and azathioprine and bleomycin) drugs, drug abusers and addicts as there have been reports that opiate drugs and intravenous and inhaled anesthetic have been shown to contribute to immunosuppression were also excluded.

Sampling

Blood samples were obtained before induction of anesthesia, 2 h after induction of anesthesia and at the end of operation. Blood hydrocortisone investigated using radioimmunoassay. Peripheral nature killer (NK) cells and T lymphocyte subpopulations (CD3, CD4 and CD8 cells) were investigated by flow cytometry.

Methodology

Identification of lymphocyte subpopulations in peripheral blood is based on recognition of their cell specific surface markers by means of labeled monoclonal antibodies. The antibody is labeled with a fluorescent tag, and in medical diagnostics, detection is mainly by flow cytometry. Chip technology and particle arrays were used. In flow cytometry, a diluted cell suspension is forced through a nozzle that causes the cells to flow single file, one cell at a time, past a light beam. Detectors are positioned to measure forward and side scattered light as well as any fluorescence emission that may be excited by the light beam. Analysis of the scattered light detects each cell and provides information on its volume and morphology, and the fluorescent detectors determine which tags, were present on the cell. Flow cytometers use multiple diodes or ion lasers as light sources with, for example, emission in the blue, green, and red thus permit the simultaneous determination of three to five different fluorescent tags on a single cell .There is electrostatic separation of homogeneous cell populations, and is termed fluorescence-activated cell sorting (FACS).

EDTA and heparin were used as the anticoagulant. Same-day analysis was done for immunophenotyping. Blood was transported and stored at room temperature.

Blood cell processing

Venous blood was collected in Ethylenediamine Tetraacetic Acid (EDTA) tubes. The blood was centrifuged to remove the

plasma, and the cell pellet was sedimented on Dextra. Samples were centrifuged for 20 min at 700 mg. The samples was re-suspended and red cells lysed with distilled water. In a peripheral blood smear, a normal lymphocyte has a large, dark-staining nucleus with little to no eosinophilic cytoplasm. In normal situations, the coarse, dense nucleus of a lymphocyte is approximately the size of a red blood cell (about 7 micrometres in diameter). Some lymphocytes show a clear perinuclear halo around the nucleus or could exhibit a small clear zone to one side of the nucleus. Polyribosomes are a prominent feature in the lymphocytes and can be viewed with an electron microscope. Flow cytometry testing is used for specific lymphocyte population counts. It is used to specifically to determine the percentage of lymphocytes that contain a particular combination of specific cell surface protein as immunoglobulins or Cluster Of Differentiation (CD) markers or that produce particular proteins. Other scientific techniques like the ELISPOT or secretion assay techniques can be used. Serum IGF-I was measured by using immunochemiluminescent technique. All patients were monitored using (ECG, NIBP, capnography, pulse oximetry, oesophageal temperature probe and peripheral nerve stimulator. Patients premedicated with midazolam (0.01-0.02mg/kg). General anesthesia was induced with propofol 2-2.5 mg/kg, fentanyl 2µg/kg and vecuronium 0.15 mg/kg body wt. Additional bolus injections of fentanyl or vecuronium were given during operation if needed. Anesthesia was maintained with sevoflurane at a concentration of 1.0% to 2% with 50% oxygen and 50 % medical air.

Blood samples were obtained from peripheral vein before induction of anesthesia, 2 h after anesthesia and at the end of procedures. Blood hydrocortisone was determined by Radioimmunoassay (RIA). Peripheral Nature Killer (NK) cells and T lymphocyte subpopulations (CD3, CD4, CD8 cells) were investigated by FACSC alibur flow cytometer and data were evaluated using CELLQuest 3. Serum Insulin-like Growth Factor (IGF)- I (113 ng/ml) is a component in the diagnosis of disorders such as ALI or ARDS. It is affected by parameters such as age, gender, ethnicity, medications, chronic illness, or assay methodologies. IGF to be measured using -- Quantitative Mass Spectrometric Immunoassay before induction and at the end of surgery and alpha -2 MG using-serum protein electrophoresis--twice before induction and 24 hours postoperatively.

Results

Statistical analysis

All statistical analysis was performed with SPSS software. P < 0.05 was considered to be statistically significant. Sample sizes equal to 30 patients in each group is sufficient for the; the distribution of the sample means is normally distributed. Therefore, the more samples one takes, the more the graphed results take the shape of a normal distribution. For continuous variables, mean ± SD are considered, nominal variables are shown as percentages (%). Median values of Ventilatory Parameters (VT, respiratory rate, peak pressure, etc) from each patient were recorded. Variables were compared using either ANOVA or Chi-square to detect potential differences between both VT groups: > 12 mL/kg PBW and < 6 mL/kg PBW.

Results are expressed as mean±SEM, as data were normally distributed. Purification and recovery of blood lymphocyte subpopulation were analysed using a one-way Analysis Of Variance (ANOVA). CD [3,4,8] were assessed using one-way repeated Analysis Of Variance (ANOVA). Wilcoxon's test and Pearson or Spearman's rank tests were also used. Continuous data are pre-

sented as the median and, and some data are presented as frequency and percentage. The Mann-Whitney test was used for analyzing continuous data and the chi-square test was used for analyzing categorical data. There was no significant difference of patients' ages, operation time, but there is significant difference regarding weight, height and gender. There was a significant difference regarding sex and obesity, as most of LTV group were obese, short and females. Most of STV group were males, non-obese and tall (Table 1).

Table 1: Demographic data

Group	n	Age y	Weight KG	OP. Time	F/M	Obesity
STV	30	20 ± 18	68 ± 9	200 ± 35	7/23	8/30
LTV	30	23 ± 19	92 ± 10	200 ± 33	22/8	19/30

Three patients (10%) in the LTV group received blood transfusion while only one case (3.3%) in STV group needed. Statistically significant differences between V_T groups were found as patients in the V_T > 12 mL/kg PBW group showed a significantly greater proportion of females and obese patients (defined as BMI ≥ 30) than patients receiving V_T < 6 mL/kg PBW (Table 1). Blood hydrocortisone level was the same just before induction, increased significantly after induction in both groups. After 2 hours its significant increase continued in LTVG till the end of the procedure while its level started to decrease in STVG. (Table 2).

Table 2: Changes in blood hydrocortisone in two group's ug/L.

Group (n=30)	Before Induction	After 30m.	2h after induction	at the end
STV	384 ± 103	550 ± 70	510 ± 82	480 ± 91
LTV	379 ± 98	566±90	690 ± 101	610 ± 84

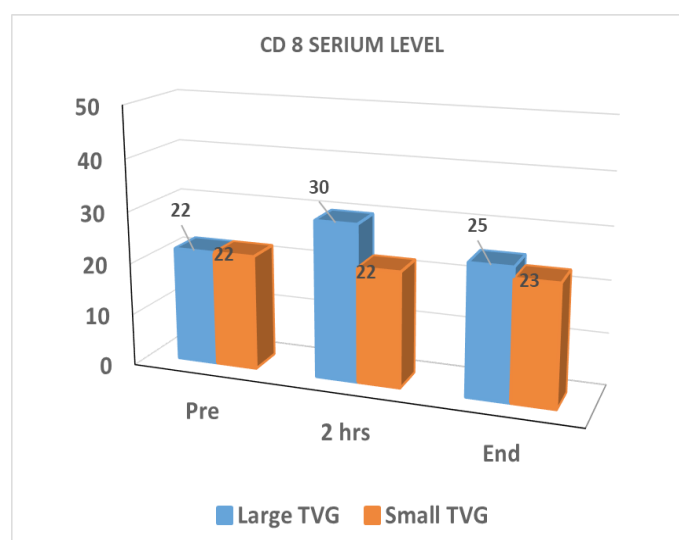


Figure 2: CD8 changes in both groups.

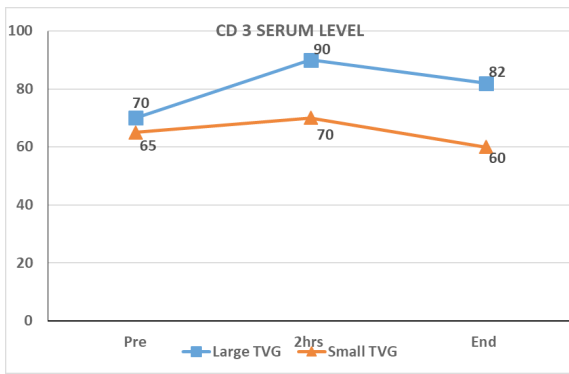


Figure 3: CD3 changes in both groups.

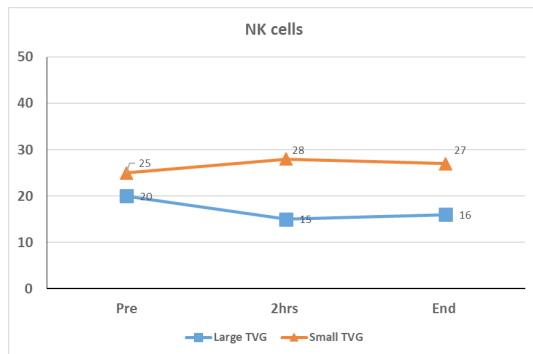


Figure 4: NK cells changes in both groups

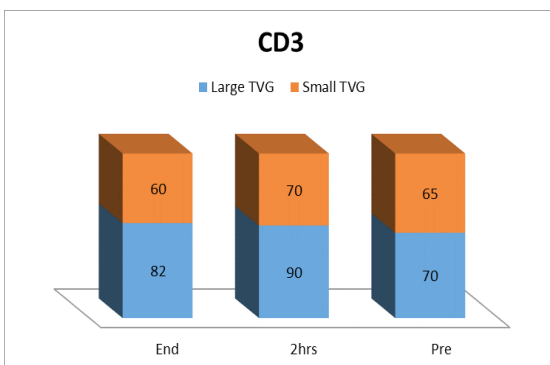


Figure 5: CD3 changes in both groups.

CD3, CD4, CD8 and CD4/CD8 ratio increased significantly after 2h and till the end of the procedures especially at LTV group.

Table 3: CD3, CD4, CD8,CD4/CD8 ratio and NK changes in both groups.

	Group (n=30)	preinduction	2 h after anesthesia	at the end of operation
CD3	STV	72.2 ± 6.5	75.6 ± 5.2	70.0 ± 6.0
	LTV	71.3 ± 5.8	88.4 ± 6.2	86.6 ± 5.9
CD4	STV	48.8 ± 6.0	45.0 ± 5.5	46.2 ± 5.3
	LTV	48.0 ± 5.8	55.1 ± 5.2	59.7 ± 5.2
CD8	LTV	22.1 ± 5.4	22.6 ± 6.1	28.0 ± 5.8
	STV	22.7 ± 5.3	29.4 ± 5.8	23.3 ± 5.8
CD4/CD8	STV	2.2 ± 0.3	1.5 ± 0.2	1.5 ± 0.2
	LTV	2.3 ± 0.3	2.9 ± 0.3	2.9 ± 0.2
NK	STV	22.3 ± 4.8	21.2 ± 4.8	20.5 ± 4.3
	LTV	22.9 ± 5.2	14.0 ± 5.6	15.2 ± 5.2

NK cells decreased significantly in LTV group compared to STV group as it started after 2 hours of induction till the end of the procedure (Table 3 and Figure 4).

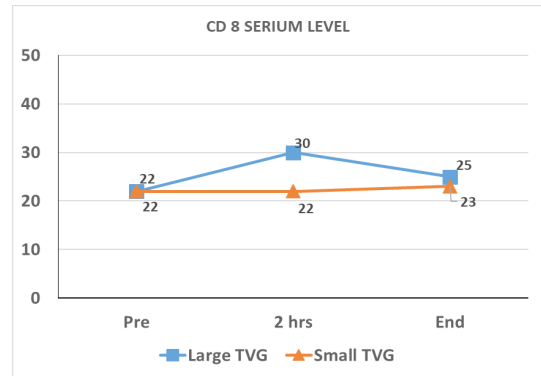


Figure 6: CD8 changes in both groups.

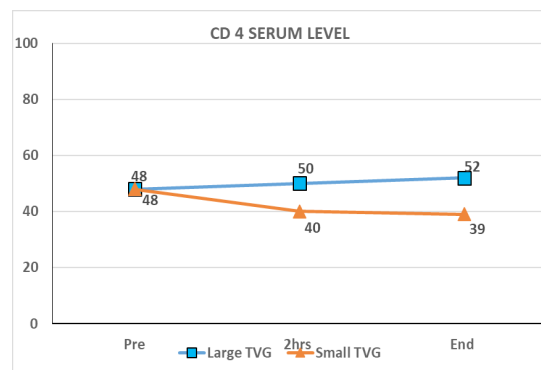


Figure 7: CD8 changes in both groups.

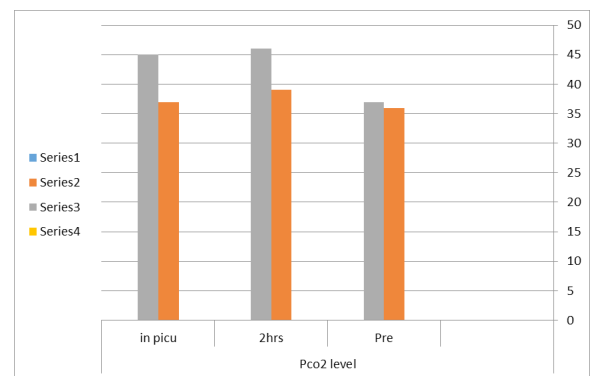


Figure 8: ECO2 in both groups. ABGs had significant hyperventilation in LTV group compared with STV group . Median range of ET CO2 was 23mmhg in LTV group vs 35 mmhg in STV group.

ABGs recorded significant hyperventilation in LTV group compared with STV group. Median range of ET CO2 was 23mmHG in LTV group vs 35 mmHG in STV group (Figure 8).

All LTVG patients were hyperventilated with PCO2 (22-27 mm Hg ± 1.4) and mild respiratory alkalosis (PH=7.29-7.31± 0.5) after 2 hours of mechanical ventilation. Nineteen (63.3%) patients in LTV group and only 2(6.6%) patients in STV group showed hyperinflation of the lungs after 2 h of induction by simple chest X-ray. After 6 hour, 15 patients of LTV group and no patients in STVG showed hyperinflated lungs (Figure 9).

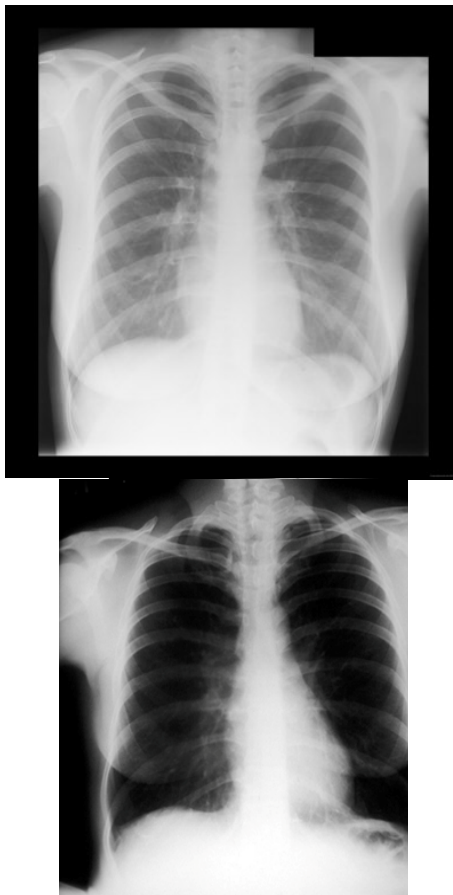


Figure 9: Chest X-ray showed hyperinflated lungs. Median value of TV in LTV group was 620ml and 480ml in STV group. Peak airway pressures were significantly high in LTV group compared to STV group. (p- value < 0.05).

The incidence of postoperative mechanical ventilation (POMV) and ICU admission was greater in patients receiving $V_T > 12$ mL/kg PBW compared with the $V_T < 6$ mL/kg PBW group. Only 2 patients in LTV group and 1 patient in STV group need PO ICU, one of the LTV group was mechanically ventilated for 24 h and weaned safely without any mortality of the 2 groups. The distribution of gender and obesity in patients requiring POMV and ICU admission was significantly different in the 2 groups. Of those 3 patients needing ICU admission, only 1 required mechanical ventilation for 24 h and had ventilatory data recorded. Median tidal volumes in the ICU were significantly smaller than those used intraoperatively (474.9 ± 70.4 vs. 530.8 ± 98.6) ($p = 0.040$). Of these had oxygenation criteria of ARDS ($PaO_2/FiO_2 < 200$) and 2 of ALI (PaO_2/FiO_2 200-300) (with radiographic criteria), no mortality during their hospital stay.

Serum Insulin-like Growth Factor (IGF)- I (113 ng/ ml) is a component in the diagnosis of disorders such as ALI or ARDS showed significant increase in LTVG at the end of surgery that explain harmful effects to lungs (Table 5).

IL-GF	Item	LTVG	STVG
	Before induction	115 ± 4	113 ± 6
	At the end of surgery	135 ± 2	117 ± 3

Discussion

Researches suggested that higher tidal volumes can injure healthy lungs, stimulate the release of inflammatory chemicals and predispose lungs to injury through ventilator-induced lung injury. Emmanuel Futier *et al* used low tidal volumes (6-8 mL/kg ideal body weight for saving people with acute respiratory distress syndrome (ARDS).

As reducing mortality by a about 20% in ARDS with Low tidal volume ventilation. Low tidal volume ventilation for ARDS has become one of the beneficial therapies in critical care medicine [14,15]. Its main disadvantages were difficulty to improve refractory hypoxemia and Co2 retention (hypercapnia) that lead to increased ICP and IOP. Others, encourage tidal volumes of 10-15 mL/kg in order to prevent atelectasis, hypercarbia and hypoxemia in surgical patients under general anesthesia. It is still common today for patients undergoing surgery to receive high tidal volumes (about 700-750 mL in the average person). Positive end-expiratory pressure (PEEP), reduces shear stress on alveoli during mechanical ventilation. Some studies and trials have not shown clear excess risks among people receiving high tidal volumes while undergoing routine surgery, and some have found evidence of preclinical ventilatory-induced lung injury, others have argued that low tidal volume ventilation may actually be harmful in surgical patients.

Low tidal volume mechanical ventilation- 6-8 mL/kg ideal body weight; PEEP 6-8 cm H2O; recruitment maneuvers every 30 minutes; that is has one difference of our study as we did not use recruitment maneuvers to avoid hypotension and increases in ICP and IOP.

Patients of some previous studies had equivalent intraoperative experiences (length of surgery, blood loss, etc.) and were treated identically during surgery (volume of fluids administered, etc.) that antagonize our results. The primary end point was reached if any of a list of major complications occurred or need for invasive or noninvasive ventilation for acute respiratory failure.

A2M is a glycoprotein that consists of 36 exons. The protein is found in serum concentrations of 2-4 mg/mL. It consists of two identical subunits, which themselves are made up of two peptide chains covalently linked by disulfide bonds. Alpha-2M is one of the acute phase proteins. Plasma levels of Alpha-2M are reduced in patients with sepsis and ALI. In our study, its level is reduced significantly after 24 hours postoperatively in LTVG that explained the cases had clinical and laboratory ALI in our study (Table 4).

A2M	Item	LTV	STV
	Before Induction	3 ± 0.3	3.5 ± 0.2
	24 hours Postoperative	1.5 ± 0.1	3.1 ± 0.2

The larger median exhaled V_T was associated with a significant increased minute volume ventilation, greater peak pressures and lower values of end-tidal CO2 partial pressure. No differences in inspired oxygen fraction or arterial oxygenation were found between both groups. Duration of the surgical procedure and the estimated blood loss were slightly greater in the $V_T > 12$ mL/kg PBW group but did not reach statistical significance.

Obesity is the most significant risk factor for a patient receiving a $V_T > 12$ mL/kg PBW. The use of blood was significant in this analysis as three patients of large TV group received blood and only one patient in STV group needed. Most patients in the $V_T < 6$ mL/kg PBW had heights > 160 cm and PBW < 77 kg, while most patients in the $V_T > 12$ mL/kg PBW had heights < 160 cm and PBW >77 kg.

Low tidal volume ventilation during surgery prevented postoperative acute respiratory failure: only one case (3.3%) of patients in the low tidal volume ventilation group required non-invasive ventilation postoperatively, compared to 3 cases (10%) LTV group. Three cases of STV group and only one case of LTV group had radiological atelectasis that needed no treatment. The hospital of stay was slightly shorter in those receiving low tidal volume ventilation. There was no apparent harm from low tidal volume mechanical ventilation. Low tidal volume ventilation for ARDS is not as a beneficial therapy, but can avoid a harmful practice that matched our results.

Emmanuel Futier et al concluded that ; low tidal volumes can be beneficial (or that high tidal volumes can be harmful) in patients without evidence of ARDS or acute lung injury [14,15] that matches the result of our study but our study conducted in a normal lungs without ARDS or ALI and we estimated more parameters e.g immunological, hormonal and mechanical effects of different TVs. In 2012, a large meta-analysis with randomized trials and observational studies showed a strong trend toward reduced mortality, lung infections, and ARDS in more than 2,000 people treated with low TV that went hand in hand with our study.

Low tidal volume ventilation appears beneficial in patients undergoing abdominal surgery [15], and high tidal volumes are more harmful than previously realized. So, low tidal volumes: 500 mL for an average-height man and 380 mL for an average-height woman is ideal as it led to, lower mortality, faster extubation rates, and a lower incidence of Ventilator Associated Pneumonia (VAP). As they use 6-8 mL/kg for initial settings with a PEEP of 5cm H₂O and adjust settings per blood gas results the only difference was that our current study did not use PEEP [14,15]. Gajic O et al found that; respiratory failure is a leading cause of postoperative morbidity and mortality in patients undergoing pneumonectomy. The authors hypothesized that mechanical ventilation with large Tidal Volumes (VTs) is associated with increased risk of postpneumonectomy respiratory failure that matches our study. Postoperative respiratory failure, defined as the need for continuation of mechanical ventilation for greater than 48h postoperatively or the need for reinstitution of mechanical ventilation after extubation [15]. So, Patients who developed respiratory failure were ventilated with larger intraoperative VT than those who did not (median, 8.3 vs. 6.7 ml/kg predicted body weight; $P < 0.001$). Larger intraoperative VT was associated with development of postoperative respiratory failure. The interaction between larger VT and fluid administration was also statistically significant. They concluded that; mechanical ventilation with large intraoperative VT is associated with increased risk of postpneumonectomy respiratory failure [14,15] that matches our study as large TV stimulate inflammatory mediators that may lead to ALI or ARDS. Two randomized controlled trials confirmed the existence of so-called ventilator-associated lung injury by showing reduced morbidity and mortality with the use of lower tidal volumes in patients with Acute Lung Injury (ALI) or its more severe form, Acute Respiratory Distress Syndrome (ARDS). While guidelines now strongly advise using lower tidal volumes in ALI/ARDS patients, at present there are no widely agreed upon guidelines for setting tidal volumes in patients who do not suffer from ALI/ARDS so, we recommend using low TV ventilation to protect the lungs. Trials on lung-protective mechanical ventilation using lower tidal volumes in patients not suffering from ALI/ARDS is important. There is a relation between the use of large tidal volumes and the development of lung injury that matches our paper to some extent.

The inconsistent results from smaller randomized controlled trials, however, do not definitely support the use of lower tidal volumes, these results oppose our results. The association with potentially injurious ventilator settings, in particular large tidal volumes, suggests that additional lung injury in mechanically ventilated patients without ALI/ARDS is a preventable complication as done in our research. More prospective studies are needed to evaluate optimal ventilator management strategies for patients not suffering from ALI/ARDS for longer periods and to investigate more parameters and more patients. The study showed that [1]; ventilation with large tidal volumes ($V_T > 12$ mL/kg PBW) may occur in 50% of surgical patients [2]; intraoperative tidal volumes do not routinely correlate with accurate predicted body weight calculations; and [3] obesity, female gender or short height are risk factors for receiving large V_T during prolonged plastic surgery. Avoidance of large TV is the most efficient strategy to prevent or treat Acute Lung Injury (ALI) or Acute Respiratory Distress Syndrome (ARDS). In patients without lung injury or risk factors for it, recent reviews recommend the use of $V_T < 12$ mL/kg PBW up to 6ml/kg. Other authors have previously observed benefits of a low V_T ventilation strategy in surgical patients without evidence of lung injury, in terms of decreased inflammation or improved outcomes that matches our study as we added 2 important indicators named ILGF and A2M [12,13]. Refractory hypoxemia leads to resistance for applying lung protection ventilation strategies in patients with ALI criteria during general anesthesia for surgical procedures so, we studied the normal lungs. The PBW formula is not an easily calculated, this explain why patients of shorter height and obese patients, with unusual height/weight proportions, are more likely affected by the unintentional use of large tidal volumes, these results correlate with the results of our study. The risk of females and/or short height for receiving large tidal volumes has been observed before in the ICU setting. Greater incidence of POMV and ICU admission and longer hospital stay with the use of $V_T > 10$ mL/kg PBW compared to the $V_T < 6$ mL/kg PBW group are explained in our study by physical and biochemical effects of the use of large TV ventilation.

The different incidence of blood transfusion within the 2 groups is explained by surgical technical challenges and miscalculation of blood loss/blood volume related to obesity than by a direct link to ventilator settings. The impact of intraoperative use of large tidal volumes on the incidence of postoperative ICU admission and ALI/ARDS needs confirmation from a multiple studies.

General anesthesia and surgical stress are known to influence patients' cellular immunity. our study investigated the effect of large vs small TV at immune response The ratio of CD4/CD8 is regarded as a common measure of immune system status in healthy individuals and also commonly assessed in the diagnosis and staging of the immune system diseases. NK cells are major cytokine (producers during bacterial sepsis and activation of NK cells improves bacterial clearance by priming macrophages to help clear a subsequent bacterial challenge [2,9,10]. In this study we have shown that two different TV Techniques: Small vs large TV that lead to immune system dysfunction during plastic surgery, as shown through the imbalance in immune cell composition of the peripheral blood, including CD3, CD4 and NK cells as well as CD4/CD8 ratio.

After anesthesia and operation we observed increase in serum blood hydrocortisone in both groups. Hydrocortisone is synthesized in the cortex of the adrenal gland, where it is re-

leased into the blood stream. The effect of endocrine stress response on anesthesia and operation are always mediated by glucocorticoid hormone such as hydrocortisone. The increase of hydrocortisone level in group of large TV anesthesia increased significantly more than in group of small TV anesthesia, indicating that small TV anesthesia attenuate the endocrine stress response better than large TV anesthesia. This increase started after induction continued after 2 hours till the end of the procedure. CD3, CD4, CD8 and CD4 CD8 ratio increased significantly in LTV group after 2 hours and till the end of the procedure. This increase explained why LTV may lead to ALI or ARDS (due to significant increase of pro inflammatory mediators e.g. CD3, CD4 and CD8). Regarding NK- cells it was significantly decreased after 2 hours of induction and continued till the end of the procedure in LTVG. In this study we also found that the immunosuppression in group of large TV general anesthesia was more significant than that in group of small TV anesthesia, it may be related to the effect of dysfunction of proinflammatory mediators release. The more significant effect on endocrine stress response and release of hydrocortisone in large TV group may take action simultaneously. There have already been investigations about the relationship between the cortisol response and immune changes in the perioperative period.

This study showed that cellular immunity was interfered during large TV anesthesia. Large TV general anesthesia has less significant suppression of cellular immunity compared with small TV general anesthesia (table 2,3 and Figure 2,3,4,5,6,7).

Shin Kurosawa and Masato Kato concluded that GA accompanied by surgical stress is considered to suppress immunity, presumably by directly affecting the immune system or activating the hypothalamic-pituitary adrenal axis and the sympathetic nervous system. Blood transfusion, hypothermia, hyperglycemia, and postoperative pain and anesthetics per se are associated with suppressed immunity during perioperative periods because every anesthetic has direct suppressive effects on cellular and neurohumoral immunity through influencing the functions of immunocompetent cells and inflammatory mediator, gene expression and secretion. Immunosuppression attributable to anesthetics, such as the dysfunction of natural killer cells and lymphocytes, may accelerate the growth of residual malignant cells, thereby worsening prognoses [16]. Wolthuis EK, et al. Studied mechanical ventilation using non-injurious ventilation settings. They concluded that; lung injury in the absence of pre-existing lung injury in healthy mice is a fact. The major difference with our study is that we conducted it on humans [17]. Copland IB, et al, studied: The early changes in lung gene expression due to high tidal volume that matched results of our study. Caruso P, et al, had a different results as they concluded that low tidal volume ventilation induces proinflammatory and profibrogenic response in lungs of rats [18].

Bregeon F, et al., supported our results as they concluded that; conventional mechanical ventilation of healthy lungs induced pro-inflammatory cytokine gene transcription [19].

Amato MB, et al, studied: The effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome that matched our results. The only difference is that they studied it in injured lungs [20].

Ranieri VM, et al, studied: The effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome. Their results matched our results

as mechanical ventilation can induce a cytokine response that may be attenuated by a strategy to minimize overdistention and recruitment/derecruitment of the lung [21]. Parsons PE, et al, studied: The lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. They found that; Low tidal volume ventilation is associated with a more rapid attenuation of the inflammatory response [22]. Sundar S, et al studied: The influence of Low Tidal Volume Ventilation on Time to Extubation in Cardiac Surgical Patients. They concluded that ; Low Tidal Volume Ventilation shorten the time to extubation as it protected lungs from hyperinflation ,ALI and ARDS [23]. Wolthuis EK, et al studied; the mechanical ventilation with lower tidal volumes and PEEP. They found that; low TV ventilation prevents pulmonary inflammation in patients without preexisting lung injury; that matched our results with only one difference; they used PEEP [24]. Michelet P, et al and Choi G, et al studied: The protective ventilation influences systemic inflammation after esophagectomy a protective ventilatory strategy decreases the proinflammatory systemic response after esophagectomy, improves lung function, and results in earlier extubation and mechanical ventilation with lower tidal volumes and PEEP that prevents alveolar coagulation in patients without lung injury that matched our results [6,32]. Fernandez-Perez ER, et al found that; intraoperative tidal volume is a risk factor for respiratory failure after pneumonectomy that matched our results [25]. Wrigge H, et al, studied; the effects of different ventilatory settings on pulmonary and systemic inflammatory responses during major surgery. They found that; mechanical ventilation with high tidal volumes and increased mediator release to inflammatory stimuli or acute lung injury [9,10]. Wrigge H, et al completed their studies as they studied: The effects of mechanical ventilation on release of cytokines into systemic circulation in patients with normal pulmonary function. They concluded that; mechanical ventilation for 1 h in patients without previous lung injury caused no changes in plasma levels of inflammatory mediators. Mechanical ventilation with high V(T) did not result in higher cytokine levels compared with lung-protective ventilatory strategies. Previous lung damage seems to be mandatory to cause an increase in plasma cytokines after 1 h of high V(T) mechanical ventilation; that antagonise our results [9,10]. Licker M, et al studied: The perioperative protective ventilatory strategies in patients without acute lung injuries. They found that; more trials are needed to answer whether a multimodal lung approach effectively prevents the formation of lung atelectasis and reduces the incidence of other pulmonary complications [26]. Levitt JE and Matthay MA tried to prevent lung injury by knowing the clinical predictors of acute lung injury for prevention and earlier recognition of ALI. So, targeting the early identification of high-risk patients and those with early acute lung injury prior to the onset of respiratory failure is the aim [27]. Wolthuis EK, et al studied: The feedback and education to improve compliance in use of lung-protective mechanical ventilation and concluded much improvement in it [2,5,17,24,32]. Esteban A, et al, studied: The characteristics and outcomes in adult patients receiving mechanical ventilation. They concluded that; Survival among mechanically ventilated patients depends not only on the factors present at the start of mechanical ventilation, but also on the development of complications and patient management in the intensive care unit.

Blum JM, et al. Studied the intraoperative Ventilator management in patients with acute Lung Injury and the use of lung protective ventilation strategies. They concluded that; The Peak

inspiratory pressures were found to be 27.87 cm H₂O on average in the non-ALI group and 29.2 in the ALI group [28]. Gajic O, et al. Studied the ventilator-associated lung injury in patients without acute lung injury at the onset of mechanical ventilation. They concluded that; Strong consideration should be given to limiting large TV, not only in patients with acute ALI but also in patients at risk for acute lung injury that matched our study [29].

Brand JM, et al. Studied the effects of general anesthesia on human peripheral immune cell distribution and cytokine production they found that; the findings suggest that general anesthesia interferes with immune cell number and immune cell response. This explain the clinically well-recognized disturbance of human immunity after surgery and general anesthesia [4]. Li T, Qiu Z, et al concluded that; there was a rapid loss of both CD4 and CD8 during the acute phase of severe acute respiratory syndrome which is a different result regarding our results. [11] Scott MJ, et al confirmed that; natural killer cell activation prepare macrophages to clear bacterial infection that decreased significantly in our study during large TV ventilation [30]. Puig NR, et al studied the effects of sevoflurane GA on immunological system in mice. They concluded that; 3 days after the anesthetic exposure, animals treated with sevoflurane modulated their peripheral blood leukocyte counts and splenic lymphoid composition, while there was no evidence of hepatic or renal toxicity [31].

Conclusion

Large TV anaesthesia induced more significant increase of proinflammatory cellular immunity mediators, cortisol level and decrease in NK cells compared to small TV anesthesia. The incidence of intraoperative ventilation with $V_T > 12$ mL/kg PBW may happen because of inaccurate estimates of predicted body weight, especially in obese patients, patients of female gender or with short stature. The incidence of unintentional or intentional use of large TV in obese, females or short patients is high. So, we recommend to use small TV ventilation in all patients under GA to avoid the side effects of LTV as NK- cell depression and increase in pro inflammatory mediators, hyperinflated lungs that may lead to barotraumas, hyperventilation and ALI or ARDS. So, small tidal volume ventilation is better tolerated by patients under general anaesthesia as large TV ventilation carries the risks of immune system dysfunction, stimulation of stress hormone release and hyperinflation hyperventilation.

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