Oxidative stress and DNA damage due to one-lung ventilation

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AIM: *One-lung ventilation (OLV) is an anesthesia technique used to provide visualization in thoracoscopic lung surgeries and increase surgical site visibility during operation. In OLV, atelectasis occurs and blood from the lung participates in circulation without receiving oxygen. We designed a prospective study on patients we implemented surgery in order to research whether OLV leads to oxidative stress and DNA damage or not.*
METHODS: It *was taken 5cc blood samples 4 times from these patients in the postoperative preparatory stage (T1), on the 60th minute after the start of OLV (T2), on the 60th minute after the termination of OLV (T3) and 24 hours after surgery (T4). Total antioxidant capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI) values were examined with regards to DNA damages in the blood samples taken.*
RESULTS: *DNA damage was statistically increased with OLV compared to baseline level ($p<0.05$) and statistically decreased in 24 hour ($p<0.05$). TAC level was statistically decreased with OLV compared to baseline level and statistically increased in 24 hour ($p<0.05$). TOS level was statistically increased with OLV compared to baseline level ($p<0.05$) and statistically decreased in 24 hour ($p<0.05$). OSI level was statistically increased with OLV compared to baseline level ($p<0.05$) and statistically decreased in 24 hour ($p<0.05$).*
CONCLUSION: To *the best of our knowledge this is the first study showing DNA damage in thoracic surgery which was operated with OLV. This DNA damage found to be decreased in first postoperative day and might be related to changes in oxidative status of this patient group.*

KEY WORDS: Oxidative stress, lung ventilation, DNA damage

Introduction

Pulmonary damage after thoracic surgery is a rarely seen complication with high mortality. Factors such as cytokine imbalance, ischemia reperfusion and one-lung ventilation (OLV) take part in the etiology apart from the surgical insult itself [1-3]. OLV is frequently applied to achieve a clear operational field in thoracic surgery and also for protecting the opposite lung in cases such lung abscess as a means of isolation, taking air leaks under control in tracheal bronchial injuries and in massive hemoptysis [4-6]. During OLV, the non-ventilated lung remains not only atelectatic but also hypoperfused because of hypoxic vasoconstriction. When resuming, two-lung ventilation, re-expansion, along with oxygen reentry through the airways, causes reactive pulmonary vascular dilatation, commencing reperfusion of the lung and thus, leads to excessive oxidative radical release [7].

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Several studies focusing on oxidative stress showed that oxidative stress contributes to lung damage after lung resection and this impact is associated with duration of OLV [5,8]. Oxidative stress has been defined as a disturbance of the equilibrium status of prooxidant and antioxidant systems in favour of prooxidation [9]. This disturbance of the prooxidant/antioxidant balance is also considered to be a causative factor underlying the oxidative damage to cellular molecules, such as DNA [10].
We aimed to investigate whether possible oxidative stress in OLV influenced DNA by measuring total antioxidant capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI) and DNA damage with this prospective study in thoracic surgery patients.

Patients and Methods

Patient selection

The Institutional Ethical Committee approved this study, which was performed in accordance with the ethical principles for human investigations as outlined by the Second Declaration of Helsinki. We recruited 40 ASA physical status I–III patients undergoing thoracotomy with various indications (hydatide cyst resection, wedge resection, decortication, etc.) under elective conditions once written informed consent was obtained. Patients who were implemented major resection (lobectomy, pneumonectomy), underwent emergency thoracotomy, transferred to ICU with mechanical ventilation, received blood transfusion were excluded from the study. Patients with recent usage of antioxidants, such as vitamin preparations, and past with infectious/inflammatory/-rheumatologic disturbances, patients with chronic liver/kidney diseases were also excluded.
Anesthesia induction was made with thiopental sodium (7 mg/kg) and remifentanil (0.5 μg/kg) and 5 to 7% desflurane was used for maintenance. Vecuronium (0.1 mg/kg) was used as muscle relaxant in both groups. After induction of anaesthesia, an appropriate size of left bronchial catheter (Broncho-Cath, Mallinckrodt) was incubated and adjusted by using a fiberoptic bronchoscope before and after turning to the lateral decubitus position. Systolic blood pressure, diastolic blood pressure, heart rate, pulse oximetry, body temperature, urine output and peak airway pressure were monitored continuously. Ventilation was delivered mechanically. When OLV was started, the nondependent lung was collapsed and opened to air with suction if necessary, and the dependent lung was ventilated at a fraction of inspired oxygen (FiO_2) of 1, a tidal volume of 8 to 10 ml/kg, a respiratory rate of 12 to 16 breaths/min adjusted to maintain the arterial carbon dioxide between 35 and 45 mmHg, and an inspiration:expiration ratio of 1:2. The concentrations of inspiratory and expiratory gas mixture (FiO_2, end-tidal CO_2, fraction of inspired desflurane and end-tidal desflurane) were continuously monitored (Capnomac Datex). Demographic data, hemodynamic data, end-tidal CO_2, peripheral O_2 saturations, anaesthesia, surgery and OLV times were recorded.

Blood sampling and laboratory analysis

Blood samples were collected in every patient following a fixed blood sampling protocol. Peripheral venous blood of 5 cm^3 was collected at each time. The first blood samples were taken in the preoperative preparatory period (T1). The second blood samples were taken on the 60th minute after starting the one-lung ventilation (T2). The third samples were taken on the 60^{th}minute after ending the one-lung ventilation and starting the two-lung ventilation (T3). The fourth blood samples were taken 24 hours after surgery (T4).TAS, TOS, OSI values were examined with regards to DNA damages and oxidative stress in the blood samples taken by using the methods explained below.

Blood collection and storage conditions

Onditionssamples were immediately transferred into heparinized tubes, stored at 2-4 8C in the dark to prevent further DNA damage, and processed within 2 h. Mononuclear leukocytes were isolated by centrifugation on Histopaque 1077 (Sigma). One milliliter amounts of heparinized blood were carefully layered over 1 ml amounts of Histopaque and centrifuged for 35 min at 500 x g at 25 8C. Each interface band containing mononuclear leukocytes was washed with phosphate-buffered saline (PBS) and collected by 15-min centrifugation at 400 x g. The resulting pellets were resuspended in PBS and cells were counted using an automatic cell counter (Abbott 3700, USA). Membrane integrity was assessed using the Trypan-Blue exclusion assay. The remaining blood was centrifuged at 1500 x g for 10 min to obtain plasma which was stored at - 80 °C prior to analysis of total oxidant status (TOS) and total antioxidant status (TAS).

Determination of DNA damage using the alkaline comet assay

The comet assay, also known as the single-cell gel electrophoresis (SCGE) assay, was performed as described by Singh et al. [11,12] with the following modifications: 10 ml amounts of fresh mononuclear leukocyte cell suspensions (roughly 20,000 cells) were mixed with 80 ml of 0.7% low melting-point agarose in PBS at 37 °C. Next, 80 ml of each mixture was layered onto a slide precoated with a thin layer of 1% normal melting-point agarose (NMA) and immediately covered with a cover

slip. Slides were held for 5 min at 4 8C to allow the agarose to solidify. After removal of cover slips the slides were immersed in freshly prepared cold (4-8C) lysing solution (2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris–HCl, 1%Triton X-100, and 10% DMSO [added just before use]; pH 10-10.5) for at least 1 h. Slides were next immersed in freshly prepared alkaline electrophoresis buffer (0.3 M NaOH and 1 mM Na^{2}EDTA; pH > 13) at 4 8C to allow DNA to unwind (40 min) and then electrophoresed (25 V/300 mA, 25 min). All manipulations were performed under minimal illumination. After electrophoresis, the slides were neutralized (0.4 M Tris–HCl; pH 7.5) for 5 min.Dried microscope slides were stained with ethidium bromide (2 mg/ml in distilled water; 70 ml/slide), covered with cover slips, and viewed by fluorescence microscopy (Olympus BX51, Japan) at 200x magnification. The microscope was capable of detecting epifluorescence and was equipped with a rhodamine filter (excitation wavelength 546 nm; barrier 580 nm). The extent of extranuclear fluorescence was scored (by eye) in 50 random cells of each sample using a scale of 0-4 as previously described by Kobayashi et al.[13.14] . Scoring was as follows: 0, no tail; 1, comet tail < half the width of the nucleus; 2, comet tail equal to the width of the nucleus; 3, comet tail longer than the width of the nucleus but not twice as long; 4, comet tail > twice the width of the nucleus (Fig. 1). This type of scoring has been shown to be as accurate that afforded by computerized image analysis. All slides were coded and were scored in a blinded manner. A visual score for each class of subjects was calculated by multiplying the percentages of cells in the various comet classes by the score for that class. The total visual comet score reflecting the extent of DNA damage was the sum of scores for all five comet classes. Thus, a total visual score could range from 0 (all undamaged) to 400 (all maximally damaged) in arbitrary units (AU).

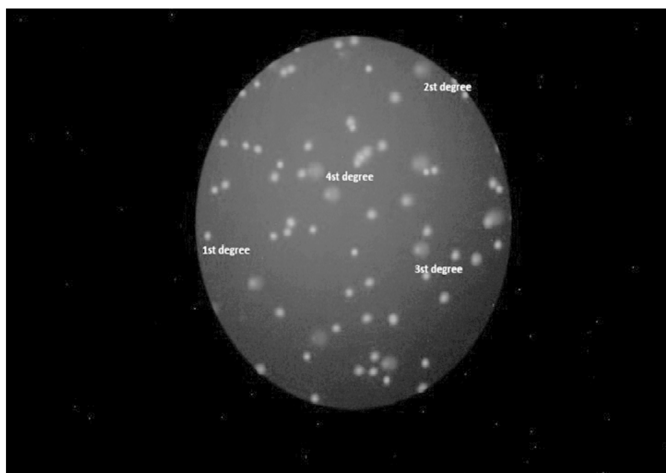

Fig. 1: 4th grade of DNA damage.

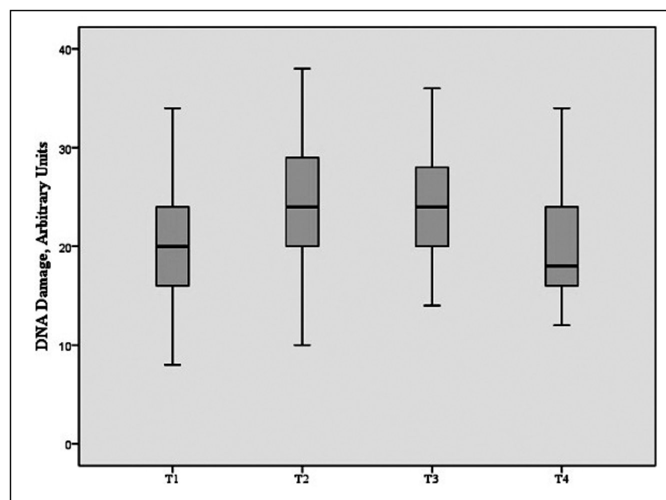

Fig. 2: The box-plot figure showing the DNA damage levels of the work groups.

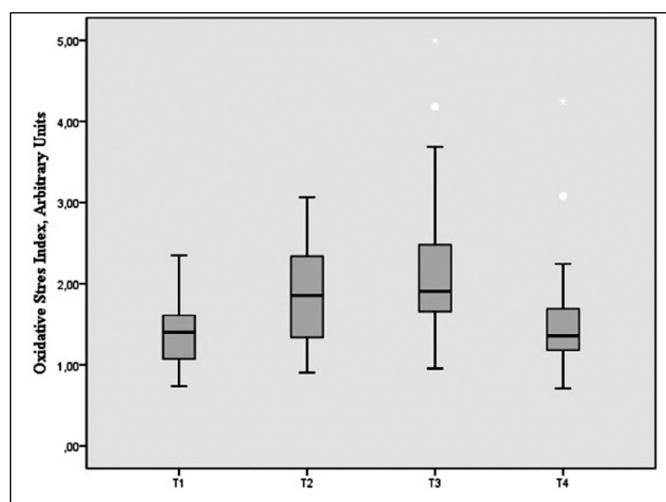

Fig. 3: The box-plot figure showing the Oxidative Stress index levels of the work groups.

Measurement of total oxidant status

Plasma TOS was measured using a novel automated method developed by Erel [15]. Oxidants present in a sample oxidize the ferrous ion of an o-dianisidine complex to ferric ion. Oxidation is enhanced by glycerol, which is abundant in the reaction medium, and the ferric ion forms a colored complex with xylenol orange under acidic conditions. Color intensity (which can be measured spectrophotometrically) is associated with the total level of oxidants present. Hydrogen peroxide is used to calibrate the assay and results are expressed in terms of micromoles of hydrogen peroxide equivalent per liter (mmol H_2O_2 equiv./l).

Measurement of total antioxidant status

Plasma TAS was measured using another novel automated method developed by Erel [16]. This involves production of the hydroxyl radical, which is a potent biological reactant. A ferrous ion solution is mixed with hydrogen peroxide. Radicals produced by the hydroxyl radical, including the brown dianisidinyl radical cation, are also potent in biological terms. Thus, it is possible to measure the antioxidative capacity of a sample in terms of inhibition of free radical reactions initiated by production of the hydroxyl radical. Variation in assay data is very low (less than 3%) and results are expressed as mmol equiv./l.

Measurement of oxidative stress index

The OSI was the TOS-to-TAS ratio, but TAS values were changed to mmol/l. Each OSI was calculated as follows: OSI (arbitrary units) = TOS(mmol H_2O_2/l)/TAS(mmol/l)[17].

Statistical Analyses

The statistical analyses were performed by using SPSS Versiyon 20.0 (SPSS Inc. Chicago USA) program. One-way variance analysis (ANOVA) was used in data where parametric hypotheses realized for multiple comparisons. The results were expressed as mean ± standard deviation (SD). The significance levels of the differences between the mean values of the groups were compared through Student's t test. P<0.05 was accepted as statistically significant.

Results

All of the patients completed the study. Age, gender, weight, height, operation, OLV, anaesthesia time were shown in Table I. Mean DNA damage, TAS, TOS and OSI levels in T1-T4 time intervals were shown in Table II.

Table I - *Demographic Characteristics.*

OLV group	Sex		
	Female	Male	Average
Number (n)	17	23	
Age (year)	58±17	52±14	55±15
weight (kg)	59±11	70±13	64±12
Height (cm)	158±8	171±7	164±7
ASA I (n)	10	14	
II (n)	6	5	
lll (n)	2	3	
Concomitant diseases + (n)	12	14	
(Hypertension and diabetes) - (n)	12	10	
OLV Time (min.)	132±14	122±16	127±11
Surgery Time (min.)	154±36	148±42	151±24
Anesthesia Time (min.)	170±26	162±12	166±14

DNA damage was statistically increased with OLV compared to baseline level (20,49±5,68 Arbitrary Unit vs. 23,71±7,01 Arbitrary Unit; p<0.05) and statistically decreased in 24 hour (23.71±7,01 Arbitrary Unit vs 20-03±5.73; p<0.05).
TAS level was statistically decreased with OLV compared to baseline level (1.05±0,12 mmol troloks Eq./L vs 0,99±0,14 mmol troloks Eq./L) and statistically increased in 24 hour (0,92±0,21 mmol troloks Eq./L vs 1.02±0,17 mmol troloks Eq./L).
TOS level was statistically increased with OLV compared to baseline level (14,61±3,95 µmol H_2O_2 Eq./L vs. 18,09±6,07 µmol H_2O_2 Eq./L; p<0.05) and statistically decreased in 24 hour (18,09±6,07 µmol H_2O_2 Eq./L vs. 15.05±4,69 µmol H_2O_2 Eq./L; p<0.05).
OSI level was statistically increased with OLV compared to baseline level (1,40±039 Arbitrary Unit vs. 1,83±0,57Arbitrary Unit; p<0.05) and statistically decreased in 24 hour (1,83±0,57Arbitrary Unit vs. 1.53±0.65; p<0.05).

Table II - *The table showing the mean values and standard deviations of the work groups (ANOVA).*

	T1	T2	T3	T4	P
DNA, Arbitrary Unit	20.49±5.68[ab]	23,71±7,01[c]	23.66±5.68[c]	20,03±5,73	0,012
TAS, mmol Eq./L	1.05±0.12[a b]	0,99±0,14	0.92±0.21[c]	1,02±0,17	0,011
TOS, µmol H_2O_2 Eq./L	14.61±3.95[a b]	18,09±6,07[c]	18.82±5.70[c]	15,05±4,69	0,001
OSI, Arbitrary Unit	1.40±0.39[a b]	1,83±0,57[b]	2.16±0.88[c]	1,53±0,65	<0,001

a. p<0.05 compared to T2
b. p<0.05 compared to T3
c. p<0.05 compared to T4

Comment

We aimed to investigate whether possible oxidative stress in OLV cause DNA damage by measuring total antioxidant capacity (TAC), total oxidant status (TOS) , oxidative stress index (OSI) and DNA damage with commet assay in thoracic surgery patients. Our results demonstrated that: (i) DNA damage was statistically increased with OLV compared to baseline level and statistically decreased in 24 hour (ii) TAS level was statistically decreased with OLV compared to baseline level and statistically increased in 24 hour (iii) TOS and OSI level were statistically increased with OLV compared to baseline level and statistically decreased in 24 hour.
Mechanical ventilation and the surgical procedure may induce alveolar and systemic inflammatory responses in thorasic surgery patients. Consequently, one lung ventilation (OLV) increases the concentrations of alveolar macrophages and granulocytes, proteins, proinflammatory cytokines, and adhesion molecules (i.e., soluble intercellular adhesion molecule-1, tumor necrosis factor (TNF), interleukin [IL] 8, and polymorphonuclear granulocyte elastase) in the alveoli of the ventilated lung.[18] 2,3,4 and also OLV shown to provoke severe oxidative stress and the degree of oxidative stress was associated with the duration of OLV [5.7]. Protracted (>1 h) OLV showed to be a potential cause for cardiovascular complications through the generation of severe oxidative stress due to lung reexpansion, and it is well known that oxidative stress could easily trigger lung damage in the case of inadequate anti-oxidant capacity [7.19]. Although these events are prevented with the action of the endogenous defense mechanisms under normal conditions, the ischemic and hypoxic damage might cause neutrophil infiltration, phospholipase activation, membrane lipid alterations, cytoskeletal dysfunction, ATP depletion, intracellular calcium accumulation in different stages of the damage [20]. In the study they performed upon the role of OLV on the postresectional pulmonary oxidative stress in patients with lung cancer, Misthos et al. determined that the pulmonary re-expansion provoked the oxidative stress, the amount and rates of the free oxygen radicals created were associated with the OLV time, and tumor resection removed a large oxidative stress from organisms[5]. And we determined in our study that the oxidative stress index values changed in the OLV period, this change had continued in the reoxygenation period, then these values decreased to basal values in the postoperative period, and this was at a statistically significant level. We think that continuing to operation with two-lung ventilation in times when olv is not needed (shortening the olv time) and the used anesthetic substance to be less toxic will reduce the dna damage. Dysregulation of the oxidative status and the the expression of inflammatory mediators results in radical scavenging of key biomolecules such as proteins, lipids (cell membranes are a common target) and DNA [21-23]. I/R-induced oxygen radical formation causes oxidative DNA damage and plays a significant role in the pathogenesis of reperfusion injury. Although DNA is a well-known target for free radical attack, little attention has been paid to the injury of DNA molecules associated with ischemia and reperfusion [6]. A variety of experimental models of ischaemic injury have also shown that free radicals induce post-ischaemic oxidative damage to DNA [15-18]. In our study, the comet assay trial was performed, to determine the DNA damage with OLV in patients underwent thoracotomy. Current data showed that oxidative status and DNA damage were altered with OLV in thoracic surgery and come to baseline levels in 24 hour. We think that oxidative stress with OLV in thoracic surgery patients might be the main mechanism of observed DNA damage in this patient group. Several limitations of the study should be considered, such as small sample size and the absence of long term clinical follow up.
In conclusion, to the best of our knowledge this is the first study showing DNA damage in thoracic surgery which was operated with OLV. This DNA damage found to be decreased in first postoperative day and might be related to changes in oxidative status of this patient group. We think that continuing to operation with two-lung ventilation in times when OLV is not needed (shortening the olv time) and the used anesthetic substance to be less toxic will reduce the dna damage. Further large scale studies are needed to evaluate the effects of this disturbance in clinical outcome.
Conflict of interest: none declared.

Riassunto

La ventilazione di un solo polmone (OLV) è una tecnica anestesiologica usata per offrire la visibilità nella chirurgia polmonare toracoscopica ed aumentare la visibilità del campo operatorio durante gli interventi. Nella OLV si determina una atelectasia ed il sangue circola nel relativo polmone senza ricevre ossigeno. Abbiamo progettato uno studio prospettico in pazienti sottoposti ad intervento chirurgico per indagare se la OLV comporta uno stress ossidativo ed un danno al DNA o meno.
Il metodo adottato è stato quello di prendere campioni di sangue di 5 cc quattro volte dopo l'inizio dell'intervento nella fase preparatoria alla OLV (T1), al 60° minuto dopo l'inizio della OLV (T2), al 60° minuto dopo il termine della OLV (T3) e 24 ore dopo il termine dell'intervento chirurgico. In questi campioni ematici si è valutata la capacità antiossidante totale (TAC), lo stato ossidativo totale (TOS), l'indice di stress ossidativo (OSI) con riguardo ai danni al DNA.
Risultati: il danno de DNA è risultato statisticamente accresciuto con OVL rispetto la livello basale ($p<0.05$) e statisticamente diminuito in 24 ore ($p<0.05$). La capacità antiossidante totale (TAC) è risultata statisticamente diminuita con OVL rispetto ai valori di base e stati-

sticamente accresciuta in 24 ore (p<0.05). Lo stato ossidativo totale (TOS) è risultato statisticamente accresciuto con OVL relativamente al valore di base (p<0.05) e statisticamente deimunuito in 24 ore (p<0.05). il livello OSI è risultato statisticamente accresciuto con OVL relativamente al valore di base (p<0.05) e statisticamente diminuito in 24 ore (p<0.05).
A nostra conoscenza questo è il primo studio che dimostra un danno del DNA in chirurgia toracica eseguita con OVL. Tale danno del DNA è risultato diminuire nel primo fiorno postoperatorio e potrebbe essere messo in relazione ad un cambiamento dello stato ossidativo di questo gruppo di pazienti.

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