Original Article

The effect of bronchoscopy on oxidative and antioxidative status

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Abstract. Hypoxemia often occurs during bronchoscopy. Pulmonologists managed it with supplement oxygen and sometimes stopping the procedure. We suggest that the main source of the reactive oxygen species is hypoxia during bronchoscopy. We investigated the alterations in oxidative and antioxidative status during bronchoscopy using oxidative stress parameters including oxidative stress index (OSI) and total oxidant status (TOS). Twenty two patients included to the study for whom bronchoscopy was performed. Twelve patients were diagnosed with lung cancer. Ten patients with normal bronchoscopy comprised the control group. Blood samples were taken just before and 1 hour after bronchoscopy. For antioxidative status, total antioxidant capacity (TAC) and total free sulfhydryl groups were determined. Indicators of oxidative stress (TOS, lipid hydroperoxides, and OSI) were statistically higher ($p<0.05$, $p<0.05$, $p<0.05$), whereas indicators of antioxidative status (TAC and free sulfhydryl) were statistically lower in the after bronchoscopy blood samples than before bronchoscopy blood samples in all patients ($p<0.05$, $p<0.05$). Before bronchoscopy, indicators of oxidative stress were higher ($p <0.001$, $p<0.05$, and $p<0.001$ respectively), and indicators of antioxidative status ($p<0.05$, $p<0.001$ respectively) were lower in the lung cancer group than control group. After bronchoscopy of lung cancer group, indicators of oxidative stress (TOS, lipid hydroperoxides, and OSI) showed significant increases ($p<0.05$, $p<0.05$, and $p<0.01$ respectively), whereas indicators of antioxidative status (TAC and total free sulfhydryl groups) ($p<0.05$, $p<0.01$ respectively) were significantly decreased than control group. We demonstrate that bronchoscopy is associated with increased oxidative stress and decreased antioxidative response through possibly caused hypoxemia.

Key words: Bronchoscopy, lung cancer, oxidative stress, antioxidants

1. Introduction

Reactive oxygen species (ROS) are produced after metabolic and physiological processes, and harmful oxidative reactions may occur during the removal of these products via enzymatic and nonenzymatic antioxidative mechanisms. Increased oxidative stress has been implicated in more than one hundred disorders (1). Clinical and experimental studies have shown that oxidative stress and lipid peroxidation are involved in the pathogenesis of asthma, COPD, lung cancer (2-8).

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Received: 17.03.2013
Accepted: 20.06.2013

Bronchoscopy visualizes the trachea, proximal airways, and segmental airways out to the third generation of branching and can be used to sample and treat lesions in those airways. It is used for visually examining abnormalities in the airways such as inflammation, tumors, bleeding and for taking tissue samples or mucus. Bronchoscopy is also used to remove any objects that block the passage of the airways. Flexible bronchoscopy is generally performed in a procedure room with conscious sedation. Flexible bronchoscopy is indicated for diagnostic or therapeutic reasons. Absolute contraindications to flexible bronchoscopy include lack of patient consent, an inexperienced operator, and a lack of facilities to handle potential complications of the procedure. Relative contraindications that increase the risk of the procedure include angina or recent myocardial infarction, unstable cardiac arrhythmia, unstable bronchial asthma, respiratory insufficiency with hypoxemia and/or hypercarbia, pulmonary hypertension, coagulopathy, renal failure, and poor patient compliance.

Hypoxemia during bronchoscopy occurs frequently. It can usually be managed by supplemental oxygen and bronchodilators or, in some cases, occasionally stopping the procedure. Hypoxia-induced reactive oxygen species in the ischemia / reperfusion injury plays an important role. Ischemia followed by reperfusion constitutes a series of events in which the capacity of the antioxidant systems might be overwhelmed by the production of oxygen free radicals (9-12).

Increases in oxidants and decreases in antioxidants may impair the oxidative/antioxidative balance towards the oxidative status (13). Chronic obstructive pulmonary disease is characterized by systemic and local chronic inflammation and oxidative stress. The sources of the increased oxidative stress in COPD patients derive from the increased burden of inhaled oxidants such as cigarette smoke and other forms of particulate or gaseous air pollution and from the increase in reactive oxygen species generated by several inflammatory, immune, and structural airways cells (2-8).

Studies investigating oxidative stress respiratory diseases yielded similar results. However, studies that related oxidant systems with bronchoscopy have not been found. In this study, we investigated the alterations in oxidative and antioxidative status during bronchoscopy using oxidative stress parameters including oxidative stress index (OSI) and total oxidant status (TOS).

2. Patients and Methods

2.1. Control and Patients

The study included 22 smokers with clinical and radiological suspicion of lung cancer (14 males, 8 females; mean age 54.7 years) for whom bronchoscopy was performed. Twelve patients were diagnosed with lung cancer. Ten patients with normal bronchoscopy comprised the control group. Exclusion criteria were the presence of other neoplastic disease, COPD, heart failure, or a recent major surgical procedure, use of antioxidant drugs, vitamin and alcohol use, concomitant inflammatory diseases such as infections and autoimmune disorders, massive hemorrhage during bronchoscopy and liver or kidney disease. Informed consent was obtained from all subjects after a full explanation of the study. Baseline demographic and clinical characteristics of all the participants were recorded.

2.1.1. Bronchoscopy procedure

All necessary measures were taken prior to bronchoscopy. Patient's illnesses, allergies and medications were recorded. Drugs such as anticoagulants and aspirin, was interrupted before bronchoscopy. All patients after physical examination, chest x-ray, pulmonary function tests, blood tests (PT, INR, Hemogram etc..), and thorax tomography were performed. Bronchoscopy was performed according to the standard protocols with fiberoptic videobronchoscop (Pentax EB1830 T3) (14). Direct Observation of Procedural Skills in all patients endorsed. Eating and drinking were stopped for eight hours before bronchoscopy in all patients. Bronchoscopy was performed with general anesthesia (with propofol). During the bronchoscopy; blood pressure, heart rate and oxygen levels were monitored. During the bronchoschopy, oxygen was not required.

2.1.2. Blood sampling

Two consecutive samples of blood were taken from each patient, just before bronchoscopy and 1 hour after bronchoscopy, respectively. Blood samples were also obtained from the control group. Peripheral venous blood samples were taken from the participants in the fasting state. Blood samples were centrifuged at 3000 rpm for 10 min, and serum was separated. The samples were stored at -80 °C until they were analyzed.

2.1.3. Measurement of total oxidant status (TOS)

Total oxidant status was measured by a most recently developed automated method whereby hydrophylic and lipophylic oxidants oxidize ferrous ion to ferric ion (15). The oxidation reaction is enhanced using glycerol and ferric ion produced makes a stable colored complex with xylenol orange dye. Hydrogen peroxide solution is used as a standard assay procedure. The assay exhibits excellent values of coefficients of variation, being less than 3%.

2.1.4. Measurement of total antioxidant capacity (TAC)

Total antioxidant capacity of serum taken before and 1 hour after bronchoscopy procedures was determined using a novel automated measurement method developed by Erel (16). In this method, the hydroxyl radical, which is the most potent biological radical, is produced. In this assay, ferrous ion solution in the reagent 1 is mixed with hydrogen peroxide in the reagent 2. The sequential radicals produced by the hydroxyl radical are also potent radicals. In this assay, antioxidative effect of the sample against potent

free radical reactions initiated by the hydroxyl radical is measured. The assay has excellent precision values, which are lower than 3%. The results are expressed as mmol Trolox equivalent/l.

2.1.5. Measurement of lipid hydroperoxide

Lipid hydroperoxide level of serum was measured by an automated method using xylenol orange (17). The method is based on a known principle: oxidation of the Fe (II) to Fe (III) by lipid hydroperoxides under acidic conditions.

2.1.6. Measurement of total free sulfhydryl groups of serum samples

Free sulfhydryl groups of serum samples were assayed according to the method of Ellman as modified by Hu et al. (18) Briefly, 1 ml of buffer containing 0.1 M Tris, 10 mM EDTA, pH 8.2, and 50 µl serum were added to cuvettes, followed by 50 µl 10 mM DTNB in methanol. Blanks were run for each sample as a test without DTNB in the methanol. Following incubation for 15 min at room temperature, sample absorbance was read at 412 nm on a Cecil 3000 spectrophotometer. Sample and reagent blanks were subtracted. The concentration of sulfhydryl groups was calculated using reduced glutathione as free sulfhydryl group standard and the result was expressed as millimolars.

2.1.7. Oxidative stress index

The percent ratio of TOS to TAC gave the oxidative stress index (OSI), an indicator of the degree of oxidative stress (13, 18-20).

2.1.8. Statistical analysis

The results were presented as mean ± standard deviation or as a percentage. Categorical variables were compared using the chi-square test. Statistical evaluations of differences before and after bronchoscopy were performed using a paired Student's t-test. For continuous variables, differences between the two groups were assessed by an unpaired t-test.

A p value of less than 0.05 was considered statistically significant. Analyses were made using SPSS 11.0 statistical software.

3. Results

Bronchoscopy procedure was completed successfully and no complications were encountered during the procedure. Clinical characteristics and risk factors of the groups are summarized in table 1. There were no significant differences between the groups with respect to age, gender, body mass index (BMI) (kg/m²), and bronchoscopy time ($p>0.05$). None of the patients had a history of regular drug use. All of the patients were heavy smokers (>20 packs/year cigarette smoking).

Indicators of oxidative stress (TOS, lipid hydroperoxides, and OSI) were higher ($p<0.05$, $p<0.05$, and $p<0.05$respectively), whereas indicators of antioxidative status (TAC and free sulfhydryl) were lower in the after bronchoscopy than before bronchoscopy in all patients ($p<0.05$, $p<0.05$ respectively) (Table 2).

Table 1. Demographic and clinical characteristics of patients

	Lung cancer group n=12	Control group n=10	p
Age (years)	56.1±5.4	51.8±7.3	p>0.05
Male (%)	66.6	60	p>0.05
Female (%)	33.3	40	p>0.05
Body mass index (kg/m²)	24.3±2.5	25.5±2.4	p>0.05
Cigarette smoking (%)	100	18.2±3.4	p>0.01
Bronchoscopy time (min)	15.3±4.2	14.1±3.9	p>0.05

Table 2. The effect of bronchoscopy on oxidative and antioxidative parameters in all patients

Variables	Before bronchoscopy	After bronchoscopy	p
TOS (µmol H_2O_2 Equiv. /L)	17.52 ± 1.91	19.22 ±1.12	p<0.05
TAC (mmol Trolox Equiv./L)	1.51 ± 0.12	1.48 ± 0.11	p<0.05
LOOH (µmol tBLOOH*/L)	8.13 ± 1.21	9.26 ± 0.88	p<0.05
Free sulfhydryl	0.42 ± 0.04	0.39 ± 0.04	p<0.05
OSI (Arbitrary Unit)	1.19 ± 0.11	1.32 ± 0.11	p<0.05

TOS=total oxidant status, TAC=total antioxidant capacity, LOOH= terty buthyl hydroperoxide, OSI=Oxidative Stress Index, p=significance.

Table 3. Comparison of oxidative stress parameters before bronchoscopy

Variables	Lung cancer group	Control group	p
TOS (μmol H_2O_2 Equiv. /L)	19.27 ± 0.81	15.33 ±2.18	p<0.001
TAC (mmol Trolox Equiv./L)	1.49 ± 0.13	1.55 ± 0.11	p<0.05
LOOH (μmol tBLOOH*/L)	9.45 ± 1.13	6.47 ± 1.32	p<0.001
Free sulfhydryl	0.39 ± 0.02	0.46 ± 0.04	p<0.05
OSI (Arbitrary Unit)	1.32± 0.12	1.07 ± 0.14	p<0.001

Table 4. Comparison of oxidative stress parameters after bronchoscopy

Variables	Lung cancer group	Control group	p
TOS (μmol H_2O_2 Equiv. /L)	21.27 ± 0.79	17.24±1.06	p<0.05
TAC (mmol Trolox Equiv./L)	1.43 ± 0.09	1.51 ± 0.08	p<0.05
LOOH (μmol tBLOOH*/L)	10.11± 0.77	7.26 ± 1.61	p<0.01
Free sulfhydryl	0.38 ± 0.04	0.42 ± 0.03	p<0.05
OSI (Arbitrary Unit)	1.41 ± 0.14	1.25 ± 0.13	p<0.01

The effect of before bronchoscopy on oxidative and antioxidative parameters in lung cancer group and control group is shown in table 3. Indicators of oxidative stress (TOS, lipid hydroperoxides, and OSI) showed significant increases in lung cancer group ($p<0.001$, $p<0.05$, and $p<0.001$ respectively), whereas indicators of antioxidative status (TAC and free sulfhydryl) were significantly decreased in lung cancer group ($p<0.05$, $p<0.001$ respectively) than control group.

The effect of after bronchoscopy in lung cancer group and control group on oxidative and antioxidative parameters is demonstrated in table 4.

4. Discussion

The main findings of the study concerning all the patients may be highlighted as follows: Indicators of oxidative stress were higher, whereas indicators of antioxidative status were lower after bronchoscopy than before bronchoscopy in all patients. Indicators of oxidative stress showed significant increases in lung cancer group, whereas indicators of antioxidative status levels were significantly decreased in lung cancer group than control group to before bronchoscopy and after bronchoscopy.

Oxidative stress is a major pathogenetic component of the airway inflammation (2). The development and progression of lung cancer and COPD have been associated with increased oxidative stress or reduced antioxidant resources. Several indicators of oxidative stress, such as hydrogen peroxide exhalation, lipid peroxidation products and degraded proteins, are indeed elevated in lung cancer and COPD patients. The fall in antioxidant capacity of blood from lung cancer and COPD patients should not only be regarded as a reflection of the occurrence of oxidative stress but also as evidence that oxidative stress spreads out to the circulation and can therefore generate a systemic effect. It has been also shown that COPD is a predisposition for lung cancer through several mechanisms including oxidative stress and oxidative stress-mediated processes such as inflammation and disruption of genomic integrity (3, 20-22).

Smoking has been implicated as the main etiologic factor for the development of lung cancer and COPD (3, 23). Smoking is a complex mixture of >4700 chemical compounds of which free radicals and other oxidants are present in high concentrations (3). Oxidative damage is prominent among the hazardous effects of smoking and entails lipid peroxidation, protein oxidation, and DNA damage (13, 15-17, 23, 24). In this study we do not know the real effect of the smoking, because all the patients and control group were smokers.

It has been known that oxidative stress is a major pathogenetic component of the airway inflammation that is characteristic for COPD (3). Oxidative radicals are known to cause oxidative damage to a number of different molecules in cellular components including membrane lipids, proteins, carbohydrates, and DNA (23, 25). Smoking contains a lot of oxidative and chemical agents, and these agents can damage the biomolecules such as protein, lipid, and DNA. It has been well known that moking causes lung cancer and COPD in which free radicals and reactive oxygen metabolites increase; however, it

is still unclear which biomolecules is more affected by these agents (21-27). Therefore, in this study we investigated the alterations in oxidative and antioxidative status by comparing the situation before the bronchoscopy and the effect of after bronchoscopy intervention.

Oxidative radicals are known to cause oxidative damage to a number of different molecules in cellular components including membrane lipids, proteins, carbohydrates, and DNA (3, 23, 25). Several indicators of oxidative stress, such as hydrogen peroxide exhalation, lipid peroxidation products and degraded proteins, are indeed elevated in COPD and lung cancer patients (3, 21-22). The fall in antioxidant capacity of blood in COPD patients should not only be regarded as a reflection of the occurrence of oxidative stress but also as evidence that oxidative stress spreads out to the circulation and can therefore generate a systemic effect (3, 21-22). It has also been shown that COPD is a predisposing factor for lung cancer through several mechanisms including oxidative stress and oxidative stress-mediated processes such as inflammation and disruption of genomic integrity (3). Indicators of oxidative stress (TOS, lipid hydroperoxides, and OSI) showed significant increases in lung cancer group ($p<0.05$, $p<0.05$, and $p<0.01$ respectively), whereas indicators of antioxidative status (TAC and free sulfhydryl) levels were significantly decreased in lung cancer group ($p<0.05$, $p<0.01$ respectively) than control group.

There are a number of reports implicating excessive oxidative stress and/or inadequate antioxidant defenses in the pathogenesis of COPD and lung cancer (22-27). In this study, we observed oxidative stress parameters (TOS, lipid hydroperoxides, and OSI) significantly increased after bronchoscopy ($p<0.05$, $p<0.05$, and $p<0.05$respectively); antioxidant parameters (TAC and free sulfhydryl) significantly decreased after bronchoscopy ($p<0.05$, $p<0.05$ respectively).

It has been suggested that hypoxia played an important role in inducing reactive oxygen species production and oxidative damage in many different diseases (9-11). A number of studies have shown that the hypoxia related to COPD and lung cancer has significant relations with oxidative stress (2, 9-11, 21, 22). However we have not seen any study that related oxidant systems with bronchoscopy. This study might be the first study in literature in terms of to show the varied cause of the oxidative and antioxidative status.

Oxidant and antioxidant systems in the literature on the application of angiography study. It has been suggested that, Angiography increased antioxidant and reduced antioxidants status (28).

Our results were demonstrated increases in oxidative stress and a decrease in indicators of antioxidative status after bronchoscopy. Recently, oxidative stress index has been often used as an indicator of oxidative stress (13, 19-22). In addition, we used TOS which is a novel method in determining oxidant status (15).

Based on our results, increased oxidants and decreased antioxidants, together with a higher OSI may imply oxidative injury to airway inflammation.

Our results provide further evidence for increased oxidative stress associated with bronchoscopy, similar to that of hypoxemia mechanism, and suggest that oxidative stress may be a common event following brief episodes of oxidative injury to airway inflammation.

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