Effects of Antioxidant Therapy on Lung Clearance, Pulmonary Function Tests and Oxidant Stress in Patients With Chronic Obstructive Pulmonary Disease

Tunçalp Demir, MD[1]; Hande Demirel İkitimur, MD[1]; Nurhayat Yıldırım, MD[1]; Özlem Özmen, MD[2]; Bedii Kanmaz, MD[2]; Hafize Uzun, MD[3]; Ezel Uslu, MD[3]

[1]*Department of Pulmonary Diseases, Istanbul University, Cerrahpaşa Medical Faculty, İstanbul, Turkey.*
[2]*Deparment of Nuclear Medicine, Istanbul University, Cerrahpaşa Medical Faculty, İstanbul, Turkey.*
[3]*Department of Biochemistry, Istanbul University, Cerrahpaşa Medical Faculty, İstanbul, Turkey.*

Abstract

To investigate the effects of antioxidant therapy on pulmonary function tests (PFTs) and oxidant-antioxidant balance on COPD patients, pulmonary function tests (PFTs), arterial blood gas (ABG) analyses, alveolo-capillary permeability measurements and plasma levels of superoxide dismutase (SOD), malondialdehyde (MDA), vitamin E, and vitamin C at the initiation of the study and after six weeks of antioxidant therapy were assessed in 13 patients with stable COPD. Significant changes were observed in the PFTs and ABG after therapy, while a slight but not significant decrease was noted in lung clearance with antioxidant therapy ($p>0.05$). MDA levels decreased from 7.7±0.8 to 6.1±0.29 nmol/mL ($p:0.000$) whereas SOD levels increased from 20.4±2.4 to 23.7±2.7 U/mL with therapy ($p<0.001$). Significant increases were observed in mean vitamin E (0.76.2 vs 0.82±7.2 mg%) ($p:0.000$) and vitamin C (4.3±0.2 vs 5.1±0.4 mg/dL) levels ($p:0.000$). Our findings indicate that antioxidant therapy as an adjunct to diet is effective on oxidant-antioxidant balance but has no demonstrable effects on PFTs and alveolo-capillary permeability.

Turkish Respiratory Journal, 2004;5:(1):22-26

Keywords: *antioxidant therapy, COPD, alveolo-capillary permeability*

Introduction

An imbalance between oxidative stress and antioxidant capacity is thought to play an important role in the pathogenesis and progression of chronic obstructive pulmonary disease (COPD) (1). Cigarette smoke is the main etiologic factor in COPD, which far outweighs any of the other risk factors. Smoking causes an oxidant-antioxidant imbalance, in part because cigarette smoke itself contains a great number of reactive oxygen species (ROS) (1014 free radicals/puff and up to 300 to 500 of nitric oxide and nitrogen dioxide) and in part because it increases the number of inflammatory cells in the alveoli (2). Oxidants, while causing direct injury to the airway epithelial cells, also lead to increments in lung connective tissue proteolysis by oxidative inactivation of antiproteases, thereby playing an important role in the pathogenesis of COPD (3).

It has recently been reported that cigarette smoke exposure results in increased alveolo-capillary permeability in humans (4). Although the reason behind the ^{99m}Tc-DTPA (diethylene triamine penta acetate) clearance increase in chronic smokers is not exactly known, it has been demonstrated that chronic ciga-

Corresponding Author: *Assoc Prof. Tunçalp Demir*
Inönü Cd İntas Çamlık Sitesi
B-Blok D 19 Sahrayıcedid
81080 İstanbul, Türkiye
Phone: +90 542 316 39 01
Fax: +90 212 632 12 16
E-mail: tuncalp@hotmail.com
drtuncalp@yahoo.com

rette smoke exposure leads to a decrease in adherence and intactness of type II alveolar epithelial cells (5). Li et al have shown that cigarette smoke-induced increase in alveolo-capillary permeability is oxidant-mediated, and that the glutathione antioxidant system has a protective role against this effect of cigarette smoke. The important roles of oxidant stress in increasing alveolo-capillary permeability and in the pathogenesis of COPD have been demonstrated by *in vivo* and *in vitro* studies (6).

In epidemiological studies, an enhancement in pulmonary functions by addition of antioxidants to the diet, such as the positive and significant relationship between vitamin C intake and FEV_1 which has been observed in the National Health and Nutrition Examination Survey (NHANES)-I (7). In a study by Dow et al, a positive correlation was detected between FVC and FEV_1 values and vitamin E and beta-carotene intake in adults (8). Addition of antioxidants (alpha-tocopherol and beta-carotene) to the diet has led to decreased levels of dyspnea in patients with COPD (9).

This present study was planned to demonstrate the effect of antioxidant therapy on oxidant stress, alveolo-capillary permeability and PFTs in patients with stable COPD.

Materials and Methods

Thirteen patients with COPD from the outpatient COPD clinic of the Pulmonary Diseases Department of Istanbul University Cerrahpaşa Faculty of Medicine were enrolled in the study. They were clinically stable cases who had suffered no acute exacerbations of COPD at least for three weeks prior to enrollment. All the enrolled patients met the European Respiratory Society criteria for the diagnosis of COPD (10). All were on a treatment regimen consisting of inhaled glucocorticosteroids, long-acting inhaled -2 agonists and sustained-release theophylline at the initiation of and throughout the study period. Written informed consent was obtained from each patient at the beginning of the study. All patients were ex-smokers. None were on continuous oxygen therapy at the time of enrollment.

Pulmonary function tests (PFTs), alveolo-capillary permeability measurements and plasma levels of arterial blood gas (ABG) analyses, and plasma levels of superoxide dismutase (SOD), malondialdehyde (MDA), vitamin E and vitamin C levels were assessed at the initiation of the study and after six weeks of antioxidant therapy.

Each patient was given an antioxidant preparation in addition to his standard therapy to be used once daily for six weeks. The ingredients of the antioxidant preparation (One-A-Day Antioxidant Plus Bayer) were; vitamin A (5000 IU), vitamin C (250 mg), vitamin E (200 IU), zinc (7.5 mg), copper (1.0 mg), selenium (15.0 mcg) and manganese (1.5 mg).

Assessment of PFTs (FVC, FEV_1, FEV_1/FVC, $FEF_{25-75\%}$, FRC, TLC, RV, RV/TLC, DLco, DLco/VA, PImax, PEmax) and reversibility assessment were performed using a Sensor Medics Vmax series 22 spirometer. The spirometry test procedure uses the forced expiratory vital capacity (FVC) maneuver, in which the subject inhales maximally and then exhales as rapidly and completely as possible. To measure the lung volumes we used the nitrogen washout method. The diffusing capacity of the lungs (DL_{CO}) estimates the transfer of oxygen from alveolar gas to the red cell. We used the single-breath method for estimating DL_{CO}. P_{Imax}, P_{Emax} were obtained, as described by Black and Hyatt. The P_{Imax} was measured following exhalation to RV and the P_{Emax} following inspiration to TLC. A positive reversibility test was defined as at least 15% or 200 ml increase in FEV_1 compared to the initial value or 12% increase compared to the predicted value after inhalation of 200 μg of Salbutamol. ABG measurements were carried out using a Rapid lab 248, Chiron/Diagnostics diagnostic device.

Nuclear Medicine Methods: A radio aerosol procedure using ^{99m}Tc-DTPA was used to evaluate the effects of a 6-week course of antioxidant therapy on the permeability of the pulmonary epithelium in COPD. Each patient was ventilated by 30 mCi ^{99m}Tc-DTPA for 8 minutes using a closed ventilation system and one image per minute was taken in posterior projection, in supine position. Image acquisition lasted 45 minutes. Computerized background corrections, taking the regions of interest of both lungs into account, were carried out. Time-activity curves were generated and activity half time in lungs ($T_{1/2}$) were calculated (11-12).

Biochemical Methods: Fasting venous blood samples, before the patients received any medication, were collected in heparin coated tubes. The tubes were promptly wrapped in aluminum foil to protect against photo oxidation. Plasma was separated by refrigerated centrifuge. To avoid interferences, iron-free tubes and deionised water were used for the assay. Samples were initially stored at –70°C and later analyzed within three weeks.

To assess the evidence of lipid peroxidation, we measured thiobarbituric acid reactive substances (TBARS), which also reflect the concentration of MDA, since the assay does not directly measure the lipid peroxidation reaction. For the assay of malondialdehyde (MDA), lipid peroxidation end product was determined as thiobarbituric acid-reactive substances (TBARS) according to a modification of the method of Buege and Aust (13). SOD activities were measured by using the nitrobluetetrazolium assay (14-15). Total plasma vitamin C concentration was measured by using the 2,4-dinitrophenylhydrazine methods of Omaye et al (16). Plasma vitamin E concentration was determined according to the method of Quaife et al (17).

For the analysis of the data, *SPSS (Statistical Package of Social Sciences) 10.0 for Windows* was used. The results were defined as mean value standard deviation (SD). Paired- samples T test was used for the comparisons of values before and after antioxidant treatment. A p value smaller than 0.05 was considered to be significant.

Table 1. Demographic characteristics of the patients	
Total number	**13**
Age (years)	66.2 ± 6.9
Smoking history (pack-years)	50.7 ± 23.9
Disease duration (years)	11.8 ± 7.4

Results

The demographic characteristics of the patients are summarized in Table 1. All patients were males. The PFTs, lung volume, diffusion capacity, respiratory muscle function and ABG mean values before and after antioxidant therapy are given in Tables 2 and 3. There were no statistically significant differences between any of the lung function test results and ABG values of the patients before and after antioxidant therapy ($p>0.05$).

Mean alveolo-capillary permeability values, calculated for each lung in each patient before and after antioxidant therapy are given in Table 4. Although a slight decrease was noted in lung clearance with antioxidant therapy, the difference did not reach statistical significance (Figure 1).

Table 5 shows mean plasma MDA, SOD, vitamin E and C levels before and after antioxidant therapy. MDA levels decreased from 7.7±0.8 to 6.1±0.29 nmol/mL (p:0.000) whereas SOD levels increased from 20.4±2.4 to 23.7±2.7 U/mL with therapy ($p<0.001$). The effects of antioxidant therapy on mean MDA and mean SOD levels are given in figures 2 and 3 respectively. Mean vitamin E levels increased from 0.7±6.2 to 0.8±7.2 mg% (p:0.000), and mean vitamin C levels increased from 4.31±0.22 to 5.09±0.44 mg/dL after therapy (p:000).

Discussion

Antioxidants may not only protect against the direct injurious effects of oxidants, but may also fundamentally alter the inflammatory events that play an important part in the pathogenesis of COPD (18).

Many studies have demonstrated an increase in the markers of oxidative stress in the airspaces, breath, blood, and urine of smokers and of patients with COPD. Increased plasma levels of products of lipid peroxidation have been reported in smokers (19).

Table 2. Pulmonary function tests before and after antioxidant therapy		
	Before Treatment*	**After Treatment***
FVC (mL)	2450 ± 941.3	2532.3 ± 933.6**
FVC (%)	69 ± 15.6	71.6 ± 16.5**
FEV_1 (mL)	1250 ± 659.1	1243 ± 619.8**
FEV_1 (%)	44.9 ± 16	45.1 ± 16.4**
FEV_1/FVC (%)	51.3 ± 14.5	49.1 ± 13.9**
TLC (mL)	6623 ± 1927	7130 ± 2223**
TLC (%)	105.9 ± 28.4	113.3 ± 30.1**
RV (mL)	3898.4 ± 1695.9	4362.3 ± 1821.9**
RV (%)	161.4 ± 65	180.8 ± 74.7**
RV/TLC (%)	57.3 ± 12.3	59 ± 12.4**
DL_{CO}, mL/mmHg/min	13.4 ± 5.1	13.1 ± 5.41 **
DL_{CO} (%)	53.4 ± 23.9	54.5 ± 22.5**
DL_{CO}/VA (mL/mHg/min/L)	3.2 ± 1.2	3.1 ± 1.3 **
DL_{CO}/VA (%)	63.8 ± 24.5	61 ± 25.4**
PI_{max} (cmH_{2O})	70.1 ± 24.7	65.4 ± 22.4**
PI_{max} (%)	65.4 ± 22.4	66.1 ± 19.9**
PE_{max} (cmH_{2O})	91 ± 23.1	90.9 ± 33.9**
PE_{max} (%)	45.3 ± 11.3	45.5 ± 17**

** $p>0.05$

(FVC: Forced vital capacity, FEV_1: Forced expiratory volume in 1 second, TLC: Total lung capacity, RV: Residual volume, *DL_{CO}: Diffusing capacity, VA: Alveolar volume, P_{Imax}, P_{Emax}: Maximal inspiratory and expiratory pressure)

Plasma levels of lipid peroxidation products, measured as TBA-MDA derivates, were higher in COPD patients compared to normal subjects (20). In our study, the MDA levels decreased statistically significantly after the patients received an antioxidant preparation for six weeks.

An increase in super oxide anion levels together with alveolar macrophage and neutrophil numbers have been detected in the broncho-alveolar lavage samples of smokers. The result of cigarette smoking and chronic neutrophil inflammation is an oxidant/antioxidant imbalance (21). Kondo and co-workers found that increased super oxide generation by alveolar macrophages in elderly smokers was associated with decreased antioxidant enzyme activities, when compared with non-smokers (22). In our study, the mean SOD le-

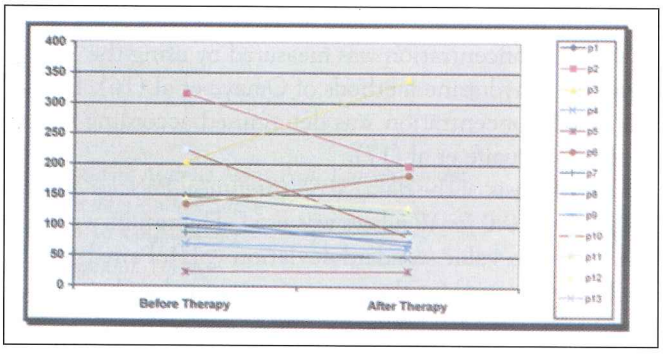

Figure 1. *The right lung clearance before and after antioxidant therapy.*

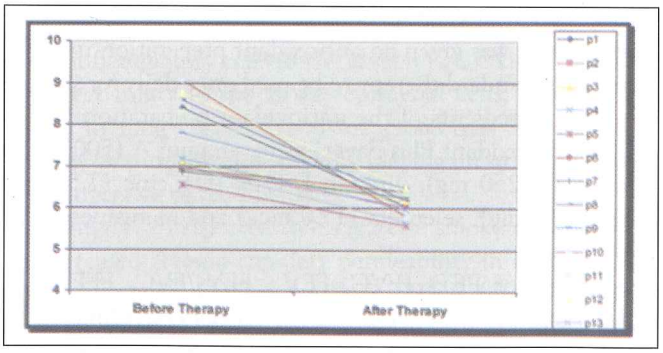

Figure 2. *MDA levels before and after antioxidant therapy.*

Table 3. Values of arterial blood gas of the patients before and after treatment

	Before Treatment*	After Treatment*
pH	7.42 ± 2.3	7.42 ± 4.2**
$PaCO_2$ (mmHg)	38.5 ± 3.3	38.7 ± 4.8**
PaO_2 (mmHg)	66.5 ± 8.8	68.8 ± 7.5**
SaO_2 (%)	93 ± 2.3	94.5 ± 2.3**
Bicarbonate (mmol/L)	25.1 ± 1.8	24.3 ± 2.9**

** p>0.05
($PaCO_2$: Partial pressure of carbon dioxide, PaO_2: Partial pressure of carbon dioxide, SaO_2: Saturation of oxygen)

vels increased statistically significantly with therapy.

The secondary antioxidant defenses include lipophilic and hydrophic antioxidants. Vitamin E, particularly the alpha-tocopherol form, is a poorly reactive and lipid soluble molecule that functions as a chain-breaking antioxidant. Following this sequence of events, the tocopherol radical reconverts to alpha-tocopherol by reaction with vitamin C (23).

There is some epidemiological evidence reporting an association between dietary intake of the antioxidants vitamin C and E and protection of lung function and chronic airway obstruction (24). There were statistically significant improvements in mean vitamin C and E levels after the administration of antioxidant therapy.

The antioxidant therapy may have a role in protecting against the development of COPD. Hence, vitamin supplementation may be a possible preventive therapy against the development of COPD (18). Supplement antioxidants have been used in several clinical trials without any demonstrable effect on lung function or symptoms (25).

Habib et al have demonstrated that addition of vitamin E to diet has no effect on initial pulmonary functions (26). On the other hand, Keskinel et al have shown that FVC, % predicted FVC, FEV_1 and % predicted FEV_1 levels tended to increase with antioxidant addition to standard therapy in patients with COPD (27). In our study, we have observed no differences between any of the pulmonary function test values before and after antioxidant therapy.

Morrison et al. (28), using ^{99m}Tc-DTPA-clearance measurement, reported that acute smoking results in an increase in epithelial permeability, which was associated with an increased number of neutrophils in broncho-alveolar lavage with some evidence of increased oxidant stress. Similar changes in vitro and in vivo can be reversed by antioxidants.

Table 4. Lung clearance values before and after antioxidant therapy

	Before Treatment*	After Treatment*
Right clearance (min)	138.6 ± 77.6	118.3 ± 83.1**
Left clearance (min)	140 ± 91.9	133.9 ± 85**

** p>0.05

In the present study, a radioaerosol procedure using ^{99m}Tc-DTPA was used to evaluate the permeability of the pulmonary epithelium in COPD patients before and after antioxidant therapy. Although a slight decrease was noted in lung clearance with antioxidant therapy, the difference did not reach statistical significance. The fact that all the patients in the study were ex-smokers may account for this finding. In a study done by Mason et al, quitting smoking was found to be associated with improvements in permeability (29). The relatively small patient group may be an important factor in our findings.

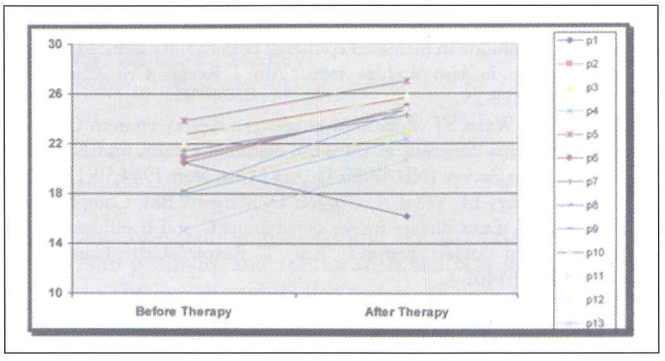

Figure 3. *SOD levels before and after antioxidant therapy.*

In conclusion, we think that antioxidant therapy may have favorable effects on oxidant- antioxidant balance in patients with COPD. However, the lack of an effect of antioxidant therapy on PFTs and on alveolo-capillary permeability can be taken as an indication of the controversial nature of its use in clinical settings.

Table 5. Effects of antioxidant therapy on oxidant-antioxidant parameters

	Before Treatment*	After Treatment*	P
MDA (nmol/mL)	7.7 ± 0.8	6.1 ± 0.3	*0.000*
SOD (U/mL)	20.4 ± 2.4	23.7 ± 2.7	*0.001*
Vitamin E (mg %)	0.7 ± 6.2	0.8 ± 7.2	*0.000*
Vitamin C (mg/dL)	4.3 ± 0.2	5.1 ± 0.4	*0.000*

(SOD: Superoxide dismutase, MDA: Malondialdehyde)

References

1. Repine JE, Bast A, Lankhorst I, and the Oxidative Stress Study Group. State of the art: oxidative stress in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1997;156:341-57.
2. Church T, and WA Pryor. Free-radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect 1981;64:111-26.
3. Rahman I, Morrison D, Donaldson K, MacNee W. Systemic oxidative stress in asthma, COPD, and smokers. Am J Respir Crit Care Med 1996;154:1055-60.
4. Jones JG, Lawler P, Crawley JCW, Minty BD, Hulands G, Veall N. Increased alveolar epithelial permeability in cigarette smokers. Lancet 1980;66-8.
5. Lannan S, Donaldson K, Brown D, and MacNee W. Effect of cigarette smoke and its condensates on alveolar epithelial cell injury *in vitro*. Am J Physiol (Lung Cell Mol Physiol) 1994;266:L92-L100.
6. Li XY, Donaldson K, Rahman I, MacNee W. An investigation of the role of glutathione in increased epithelial permeability induced by cigarette smoke *in vivo* and *in vitro*. Am J Respir Crit Care Med 1994;149:1518-25.
7. Schwartz J, Weiss ST. Relationship between dietary vitamin C intake and pulmonary function in the First National Health and Nutrition Examination Survey (NHANES I). Am J Clin Nutr 1984;59(1):110-4.
8. Dow L, Tracey M, Villar A, Coggon D, Margetts BM, Campbell MJ, Holgate ST. Does dietary intake of vitamins C and E influence lung function in older people? Am J Respir Crit Care Med 1996;154(5):1401-4.
9. Rautalahti M, Virtamo J, Haukka J, Heinonen OP, Sundvall J, Albanes D, Huttunen JK. The effect of alpha-tocopherol and beta-carotene supplementation on COPD. Am J Respir Crit Care Med 1997;156(5):1447-52.
10. Siafakas NM, Vermeire P, Pride NB, Paoletti P, Gibson J, Howard P, Yernault JC, Decramer M, Higenbottam T, Postma DS, Rees J. ERS Consensus Statement: Optimal assessment and management of chronic obstructive pulmonary disease (COPD). Eur Respir J 1995;8:1398-420.
11. Wells AU, Hansell DM, Harrison NK, Lawrence R, Black CM, du Bois RM. Clearence of inhaled 99m Tc-DTPA predicts the clinical course of fibrosing alveolitis. Eur Respir J1993;6(6):797-802.
12. Fanti S, DeFabritiis A, Aloisi D, Dondi M, Marengo M, Compagnone G, Fallani F, Cavalli A, Monetti N. Early pulmonary involvement in systemic sclerosis assessed by 99m Tc-DTPA clearence rate. J Nucl Med1994;35(12):1933-6.
13. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978;12:302-10.
14. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34:497-500.
15. Sun L, Peterson TE, Mc Cormick ML, Oberley LW, Osborne JW. Improved superoxide dismutase assay for clinical use. Clin Chem 1989;35:1265-6.
16. Omage ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods Enzymol 1979;6:3-13.
17. Quaife ML, Scrimshaw NS and Lowry OH. A micromethod for assay of total tocopherols in blood serum. J Biol Chem 1949;180:1229-35.
18. MacNee W. Oxidants/antioxidants and COPD. Chest 2000;117:303-17.
19. Marrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LJ. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. N Engl J Med 1995;332:1198-1203.
20. Taylor JC, Madison R, Kosinska D. Is antioxidant deficiency related to chronic obstructive pulmonary disease? Am Rev Respir Dis 1986;134:285-9.
21. Paredi P, Kharitonow SA, Leak D, Ward S, Cramer D, and Barnes PJ. Exhaled ethane, a marker of lipid peroxidation, is elevated in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2000;162:369-73.
22. Kondo T, Tagami S, Yoshioka A, et al. Current smoking of elderly men reduces antioxidants in alveolar macrophages. Am J Respir Crit Care Med 1994;149:178-82.
23. Housset B, Maitre B. Is There a Place or a Future for Antioxidant Therapy in COPD? In: Similowski T, Whitelaw WA, Drenne JP; eds. Clinical Management of Chronic Obstructive Pulmonary Disease New York: 2002:245-53.
24. Schunemann HJ, McCann S, Grant BJ, Trevisan M, Muti P, Freudenheim JL. Lung function in relation to intake of carotenoids and other antioxidant vitamins in a population-based study. Am J Epidemiol 2002:1;155(5):463-71.
25. Rahman I, MacNee W. Role of oxidants/antioxidants in smoking-induced lung diseases. Free Radic Biol Med 1996;21(5):669-81.
26. Habib MP, Tank LJ, Lane LC, Garewal HS. Effect of vitamin E on exhaled ethane in cigarette smokers. Chest 1999;155:684-90.
27. Keskinel I, Demir T, Aydemir A, Serdaroglu E, Yildirim N, Sahin B. The effect of antioxidant therapy on pulmonary functions and arterial blood gases in COPD. Eur Resp J 2001;18 (suppl 33):215s (abstr).
28. Morrison D, Rahman I, Lannan S, MacNee W. Epithelial permeability, inflammation, and oxidant stress in the air spaces of smokers. Am J Respir Crit Care Med 1999;159:473-9.
29. Mason GR, Uszler JM, Effros RM, E Reid BA. Rapidly reversible alterations of pulmonary epithelial permeability induced by smoking. Chest 1983;83:6-12.