Oxidative Stress in Smokers and COPD

KOAH VE SİGARA İÇENLERDE OKSİDATİF STRES

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Summary

There are numerous reports of increased oxidative stress in smokers and in patients with COPD. Cigarette smoke contains abundant amounts of oxygen-based free radicals. In addition, many studies suggest that phagocytes in smokers release increased amounts of reactive oxygen species under certain conditions. The levels of lipid peroxidation products are increased in plasma of patients with acute exacerbations of COPD compared to healthy subjects. There is limited information on the lung antioxidant defences in smokers and COPD. Studies have shown that some antioxidants are decreased in COPD. Thus, in general, it appears that an imbalance between oxidants and antioxidants occurs in cigarette smokers that favours an excess oxidant stress and may play an important role in the pathogenesis of COPD.

Key Words: Oxidative stress, Smokers, COPD

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Özet

KOAH ve sigara içenlerde oksidatif stresin arttığını bildiren birçok çalışmalar vardır. Sigara dumanı sayısız oksijen kaynaklı radikaller içerir. Bununla beraber çoğu çalışmalar belirli durumlarda sigara içenlerin fagosit hücrelerinde serbest radikal üretiminde artma meydana geldiğini göstermişlerdir. Sağlıklı insanlar ile karşılaştırıldığında akut ataklı KOAH hastalarında lipid peroksidasyon ürün düzeyleri artmıştır. Sigara içen ve KOAH'lılarda akciğerlerdeki antioksidan savunma sisitemi hakkındaki bilgiler kısıtlıdır. Çalışmalar KOAH'da birkısım antioksidanların azaldığını göstermişlerdir. Böylece sigara içenlerde aşırı oksidan strese yol açan bir oksidan-antioksidan dengesizliği oluştuğu ve bunun da KOAH patogenezinde önemli bir rol oynayabileceği düşünülebilir.

Anahtar Kelimeler: Oksidatif stres, Sigara içenler, KOAH

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Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality among the adult population. The estimated prevalance in the United States is 6% of adult white males and 3% of adult white females. According to the European Respiratory Society (ERS) consensus statement, COPD is defined as a disorder characterized by reduced maximum expiratory flow and slow forced emptying of the lungs (1).

Recently investigators questioned the role of oxidant/antioxidant in the airways. An imbalance between oxidants and antioxidants has been proposed in the pathogenesis of COPD. Respiratory tract is constantly exposed to the effects of oxidation. As well as oxygen, inhaled gases, such as O_3, NO_2, SO_2 and cigarette smoke have a strong oxidative effect (2).

Chemistry of Free Radicals

Free radicals are also known to play a role in chemical toxicity, cardiopulmonary complications, cancer, radiation injury and inflammation. Not only is the occurrence of free radical species in biological systems now established, but their involvement in both health and disease is undisputed. Endogenous sources of free radicals are the numerous enzyme and non-enzyme systems located in the subcellular membranes, cell cytoplasm, plasma and blood cell elements (3).

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A free radical is defined as a molecule containing one or more unpaired electrons. Molecular oxygen (O_2) contains two unpaired electrons and is therefore a diradical. Reduction of O_2 by 1,2, or 3 electrons, results in the formation of superoxide anion (O_2.-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH.), respectively (4).

Superoxide is relatively unreactive toward most biological substrates and very rapidly and spontaneously (or enzymatically) dismutases to yield hydrogen peroxide (H_2O_2) and oxygen O_2 (5).

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Although hydrogen peroxide is a more powerful oxidant than is O_2^- , it is relatively unreactive toward most biologic substrates unless present in unphysiologically high concentrations. It does possess several different biological properties in vitro. H_2O_2 is the immediate precursor of two more potent oxidants, hypochlorous acid (HOCl) and OH. (4). It is known that O_2^- and H_2O_2 may interact with low molecular weight iron (Fe) chelates to yield the highly reactive hydroxyl radical (OH.) via the superoxide-drives Fenton reaction(5).

$$O_2^- + Fe^{+3} \rightarrow O_2 + Fe^{+2}$$

$$H_2O_2 + Fe^{+2} \rightarrow OH^- + OH^{\cdot} + + Fe^{+3}$$

Recent data suggest that neutrophils produce very little OH. in vitro; however, O_2^- will in fact release redox active Fe from Fe strorage proteins such as ferritin, thereby providing the catalyst for OH. formation. Hydroxyl radical is an extremely reactive species, reacting immediately with virtually all known biomolecules at diffusion limited rates of reactions. Thus, it is very short lived and will react at the site where it is formed. Hydroxyl radicals have been shown to peroxidize lipids, oxidize proteins, and promote DNA strand scission. In addition to these classical reactive oxygen metabolites, activated neutrophils and monocytes secrete the hemoprotein myeloperoxidase into the extracellular medium where it catalyzes the oxidation of Cl^- by H_2O_2 to yield hypochlorous acid (HOCl) (5).

$$H_2O_2 + Cl^- + H^+ \rightarrow HOCl + H_2O$$

Hypochlorous acid possesses the two oxidizing equivalents of H_2O_2 and is approximately 100-1000 times more toxic than either O_2^- or H_2O_2 (5).

Effect of free radicals: Free radicals affects various process such as proteins, nucleic acids and DNA, membran lipids and cytosolic molecules (6). Free radical attack on membrane polyunsaturated fatty acids (PUFA) has been the focus of a great deal of work in trying to understand the role of lipid peroxidation as a mechanism of injury during oxidant stress has revealed that free radical attack on proteins is also quite common and may be very important in perturbing the molecular biology of the cell. DNA damage appears to be an early event in radical induced injury, with activation of a polysynthetase enzyme that polymerizes ADP-ribose residues from NAD. This activation can result in cellular depletion of nicotinamide and adenine nucleotides (7).

A major site of free radical attack is on PUFA in cell membranes, producing lipid peroxidation which generates hydroperoxides and long lived aldehydes. The end products of these reactions are malondialdehyde, ethene, and pentene. Levels of lipid peroxides in plasma and BAL, measured as thiobarbituric acid reactive substances (TBARS), which are significantly increased in healthy smokers and patients with acute exacerbations of COPD compared with healthy nonsmokers (8).

Antioxidant response: Under normal conditions free radicals are effectively cleared by antioxidant enzymes (9). The antioxidant mechanisms can be defined as any cell process that 1- prevents the formation of free radicals, 2- converts oxidants to less toxic species, 3- compartmentalizes reactive species away from vital cellular structures, or 4- repairs molecular injury include by free radicals (10). These defenses consist of both enzymatic and nonenzymatic antioxidants (Table 1) (5). Thus, maintaining adequate antioxidant status may provide a useful approach in attenuating the cellular injury and dysfunction observed in some inflammatory disorders. Because of the apparent importance of reactive oxygen species as toxins, mediators, and modulators of inflammatory gene activation, a number of synthetic antioxidants have been developed as potential therapeutic agents for a number of different disease states (Table 2) (5).

Table 1. Enzymatic and nonenzymatic antioxidants

Enzymatic	Nonenzymatic
Superoxide dismutase	Albumin
Catalase	Ascorbic acid
Glutathione peroxidase	Bilirubin
	Carotenoids
	Ceruloplasmin
	Glucose
	Haptoglobulin
	Hemopexin
	Pyruvate
	Sulphydryl groups
	Alpha-tocopherol
	Transferrin
	Ubiquinol-10
	Uric acid

Table 2. Synthetic antioxidants

Acetylcysteine
Calcium channel blockers
Ebselen
Etoposide
Glutathione esters
Lazaroids
Mesalazine
Nitecapone
Nitroxides
Penicillamine
Tamoxifen

Oxidative Stress in Smokers and Copd

The role of oxidants in the development of empyhsema and COPD has been extensively discussed in the last two decades (11). An increase in the concentration of reactive oxygen species is reported in the lungs of patients with COPD (2). Destruction of lung tissue or matrix components by oxidants is thought to be mediated by oxidative alteration of macromolecules, which are essential for the structural integrity of the lung (11).

There is evidence of increased neutrophil sequestration in the pulmonary microcirculation in smokers, in COPD. Circulating leukocyts in smokers and COPD have an enhanced oxidative burst (12). Cigarette smoke causes enhanced recruitment of mononuclear phagocytes polymorphonuclear leucocytes to the lower airways. These cells have altered oxygen metabolism and release more H_2O_2 and other reactive oxygen species than phagocytes from nonsmokers. These data, as a whole, suggest that high concentrations of H_2O_2 can occur in the alveolar lining fluid of cigarette smokers(13). Cigarette smoke has been implicated as a major risk factor in COPD. Free radicals and oxidative stress have been emphasized in the adverse biologic effects of cigarette smoke (14). Cigarette smoke is a rich source of oxidants (Figure 1,2).

Also Pryor and associates have identified two different populations of free radicals, one in the tar and one in the gas phase, in sigarette smoke. The principal radical in the tar phase, aquinone/hydroquinone complex, is capable of reducing molecular oxygen to superoxide radicals. The gas of cigarette smoke contains small oxygen carbon centred radicals that are much more reactive than are the tar phase radicals. In smokers, the cumulative smoking history is highly correlated with both leukocytosis and elevation acute phase reactants. This reflects a smoking induced inflammatory response with increasing accumulation of alveolar macrophages and neutrophils in the lung. In addition, smoking causes an increase in oxidative metabolism of macrophages and neutrophils (15).

The increased oxidative metabolism of phagocytes is accompanied by increased generation of reactive oxygen species, such as H_2O_2, OH.and O_2^-. Furthermore smokers have higher neutrophil myeloperoxidase activity than smokers (15).

The iron is crucial in oxidative stress, and its delocalization from iron iron-binding proteins such as ferritin may be regarded as a focal point in tissue oxidant damage (14). Within cell, most iron is unavailable for participation in free radical reactions because it is bound to ferritin, which possesses 24 subunits and 4,500 metal binding sites (16). Cells contain a transition pool of intracellular iron from which iron is withdrawn to synthesize iron proteins. The size and chemical nature of this nonprotein bound iron pool is not well established, but current models indicate that iron ions are attached to phosphate esters, organic acids and membrane lipids. In this form, the iron is available for free radical reaction (10).

Disrupted cells in regions of tissue inflammation release iron bound to ferritin and presumably

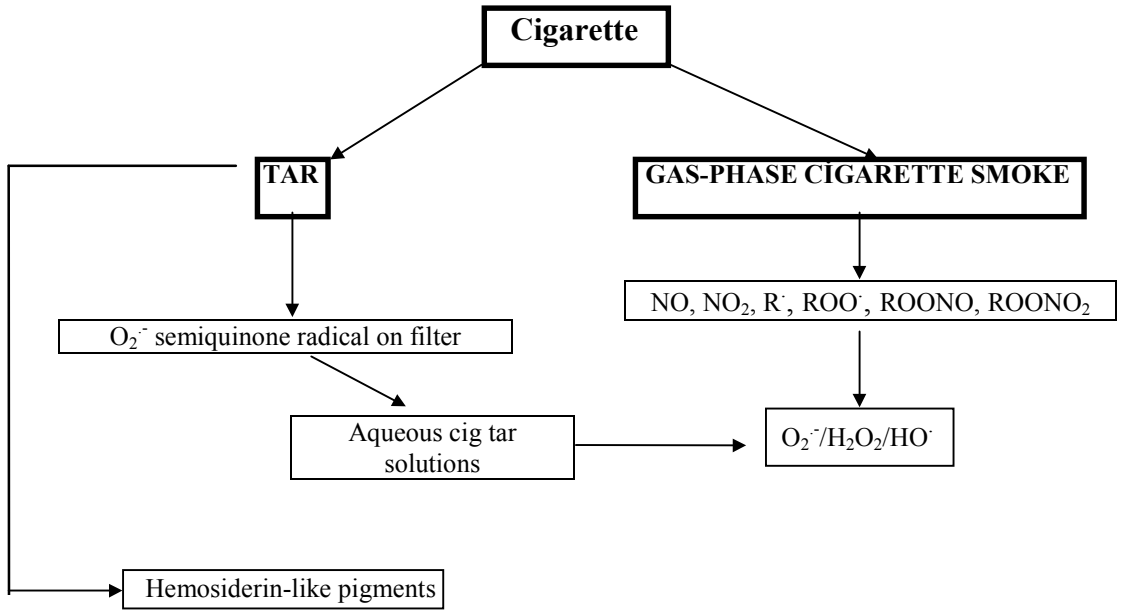

Figure 1. Components of cigarette smoke.

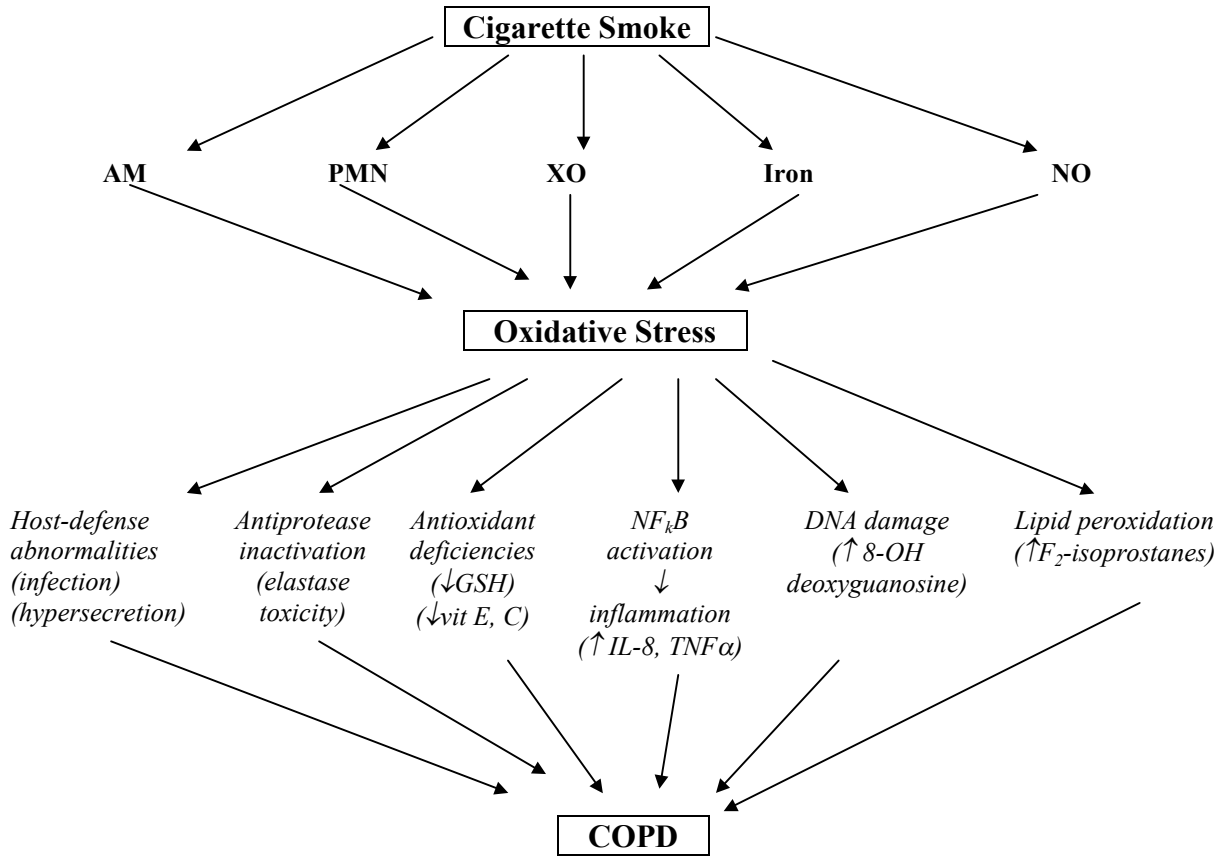

Figure 2. Cigarette smoke, oxidative stress, and COPD.

free iron from the transition pool. Ferritin iron can subsequently mobilize from the protein as the free ion after interacting with ascorbic acid, organic radicals, or O_2^- released from phagocytes and can thereby promote iron-catalyzed lipid peroxidation and generation of OH.(10). The contribution of iron has become increasingly meaningful in understanding the development of COPD. Iron concentrations increased in alveolar macrophages (AM) of cigarette smokers, and lung lining fluids obtained from cigarette smokers contained substantially more iron than specimens from nonsmokers. The source of the increased iron in lungs of smokers is unknown, but each cigarette contains 0.042µg of iron. AM from cigarette smokers also released more iron than AM from nonsmokers in vitro (16). Transferrin is the principal iron-binding protein in serum, but it also is present in airway mucosa and alveolar lining fluids. Transferrin functions as an antioxidant by tightly binding extracellular iron and thereby inhibiting oxidant-induced lipid peroxidation both in serum and in the lower respiratory tract (17). Futhermore, these observations may also suggest a role for iron in the pathogenesis of emphysema (10).

In normal lungs there are 50-70 inflammatory cells per alveolus; more than 80% are AM and less than 1% are neutrophils. In cigarette smokers the numbers of AM increase by at least 2-4 times and neutrophils by 10 times (8). AM have been implicated in the pathogenesis of centrilobuler emphysema related to cigarette smoking. The prevailing hypothesis is that emphysema occurs because lung PMNL or macrophage release proteolytic enzymes that destroy the connective tissue matrix of the lung. Supporting a role for AM are studies that showed that AK recovered from smokers contain and release more proteolytic enzymes that proteases from AM can damage lung elastin, and that protease-mediated damage to lung elastin leads to physiologic and pathologic changes that are characteristic of emphysema induced by cigarette smoke (18). In response to cigarette smoking, alveolar macrophages exhibit increases in total superoxide dismutase (SOD) and catalase activities and GSH levels when compared with nonsmokers (19).

Oxidants, whether inhaled or generated from leucocytes, can inactivate the major antiprotease alpha$_1$- proteinase inhibitor (alpha$_1$ PI) by oxidation of its active site in the airways. This diminishes the binding of alpha1 PI to elastase, hence reducing its inactivation and allowing it to bind to and destroy elastin, leading to emphysema. Although this hypothesis is supported by in vitro studies, it has been more diffucult to demonstrate convincingly oxidative inactivation of alpha$_1$ PI in vivo (8).

In view of normal oxidative burden within the lungs, pulmonary tissues are well equipped with a variety of both intracellular and extracellular antioxidant defence systems, whose concerted activities are vital to the maintenance of cellular integrity (9). Normal lung epithelial lining fluid (ELF) contains several antioxidant molecules, including glutathione (GSH), catalase, ceruloplasmin, transferrin, ferritin, ascorbate, and vit E. Relatively high GSH concentrations have been observed in the ELF of normal smokers compared with that of nonsmoking (20).

Some examples of both antioxidant decreases and increases include the following observations. In a study, investigators found that SOD, catalase, glutathione(GSH) and vit E levels were significantly lower in COPD patients as compared with controls(21). Another concluded that erythrocytes from some smokers had decreased G6PD and glutathione peroxidase(GPX) activity and were more susceptible to lipid peroxidation in vitro than RBCs from nonsmokers (16).

Rahman et al showed that plasma antioxidant capacity was low in patients presenting with acute exacerbations of COPD. In addition, they found that antioxidant activity was markedly reduced in healthy chronic smokers as compared with healthy nonsmokers (12). Stites et all reported that transferrin concantrations in lavage fluid also were decreased in COPD patients when normalized for lavage fluid protein content (17).

In addition, cigarette smoking has been associated with decreased plasma ascorbate, plasma ß-carotene, and vit C levels. Establishing the relationship between decreased antioxidant capacity and smoking remains difficult because many confounding variables, such as life-style, diet, and social class, may alter both smoking and changes in antioxidant levels (16).

A number of studies have revealed increased antioxidants in cigarette smokers. Hilbert et al showed that catalase and GPx activities were higher in BAL cells from smokers compared with nonsmokers. Likewise, vit E and ß-carotene concentrations were markedly higher in smokers lung lavage cells (19). For example vit E and C levels were increased in the plasma and internal mammary arteries of cigarette smokers compared with nonsmokers, and the smokers with higher vit C levels had lower levels of lipid peroxidation (16). Some investigators showed that increased levels of the antioxidant ceruloplasmin occured in the serum of cigarette smokers (8). The important thiol antioxidant GSH is increased in the epithelial lining fluid in the airways of chronic smokers which is related to humoral markers of inflammation. The reason for the increase in concentration of GSH in the BAL of smokers is not known, but it may be due to cell rupture leading to passive release of GSH into the extracellular space, specific triggers to cells such as type II pneumocytes or macrophages to increase synthesis and release of GSH, or to plasma exudation as a result of increased alveolar-capillary permeability due to inflammation (8).

Antioxidant Therapy in Copd

N-acetylcysteine (NAC) is the most widely investigated drug with antioxidant properties that has been used in both experimental and clinical settings which are relevant to COPD (16). Therapeutically NAC is used as amucolytic agent and as an antidote against drug-induced hepatotoxicity. In many tissues and cells, NAC is easyly deacetylated to form cysteine, which efficiently supports GSH biosynthesis (22). The potentially beneficial antioxidant effect is suggested because NAC decreased H_2O_2-induced damage to epithelial cells in vitro. In addition, NAC treatment reduced cigarette smoke-induced abnormalities in PMN, AM, fibroblasts, and epithelial cells in vitro (22,23).

The potent antioxidant and membrane stabilizing effects of vit E and its relative nontoxicity suggest that it would be an ideal pharmacologic agent to enhance pulmonary antioxidant mechanisms. This antioxidant capacity has been demonstrated in vitro with cultured endothelial cells that undergo less injury after exposure to stimulated neutrophils or H_2O_2 in the presence of supplemental vit E (10).

In summary, there is strong evidence that increased levels of oxidants, either externally administered by cigarette smoke or internally generated by phagocytes, play an important role in the development of COPD. Unfortunately, because of the great variabillty that exists in the individuals who smoke and difficulties in measuring oxidative status many challenges remain understanding, treating, and preventing COPD.

REFERENCES

1. Siafakas NM. Pathology and pathophysiology of COPD-introduction.Eur Respir Rev 1997; 7:43: 107-8.
2. Bast A. Oxidants and antioxidants in the lungs. In: van Herwaarden CLA et all (eds);COPD: diagnosis and treatment. Amsterdam: Excerpta Medica, 1996: 33-9.
3. Singal PK, Petkau A, Hrushovetz S, Foerster J. Free radicals in health and disease. Molecular and Cellular Biochemistry 1988; 84: 121-2.
4. Terada LS, Repine JE. Oxygen radicals and lung disease. in: Fishman AP. editors.Update: Pulmonary Diseases and Disorders, Philadelphia: Mc Graw-Hill, 1992: 159-75.
5. Conner EM, Grisham MB. Inflammation, free radicals, and antioxidants. Nutrition 1996; 12(4): 274-7.
6. Freeman BA, Crapo JD. Biology of disease; Free radicals and tissue injury. Lab Invest 1982; 47(5): 412-26.
7. Jenkinson SG. Free radical effects on lung metabolism. Clin in Chest Med 1989; 10(1): 3747.
8. Rahman I, Macnee W. Oxidant/antioxidant imbalance in smokers and chronic obstructive pulmonary diseases. Thorax 1996; 51: 348-51.
9. Cotgreave IA, Moldeus P. Lung protection by thiol-containing antioxidants. Bull Eur Physiopathol Respir 1987; 23: 275-7.
10. Heffner JE, Repine JE. Pulmonary strategies of antioxidant defense. Am Rev Respir Dis 1989; 140: 531-54.
11. Jörres RA, Magnussen H. Oxidative stress in COPD. Eur Respir Rev 1997; 7(43): 131-5.
12. 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.
13. Nowak D, Antczak A, Krol M et all. Increased content of hydrogen peroxide in the expired breath of cigarette smokers. Eur Respir J 1996; 9: 652-7.
14. Lapenna D, Gioia SD, Mezzetti A et all. Cigarette smoke, ferritin, and lipid peroxidation. Am J Respir Crit Care Med 1995; 151: 431-5.
15. Chow CK. Cigarette smoking and oxidative damage in the lung. Annal New York Academy of Scien 1994; 5: 289-98.
16. Repin JE, Bast A, Lankhorst I et all. Oxidative stress in COPD. Am Respir Crit Care Med 1997; 156: 341-57.

17.Stites SW, Nelson ME, Wesselius LJ. Transferrin concentrations in serum and lower respiratory tract fluid of mechanically ventilated patients with COPD or ARDS. Chest 1995; 107: 1681-85.

18.Hoidal JR, Fox RB, LeMarbe PA et al. Altered oxidative metabolic responses in vitro of alveolar macrophages from asymptomatic cigarette smokers. Am Rev Respir Dis 1981; 123: 85-9.

19.Hilbert J, Mohsenin V. Adaptation of lung antioxidants to cigarette smoking in humans. Chest 1996; 110: 916-20.

20.Melloni B, Lefebvre MA, Bonnaud F et al. Antioxidant activity in bronchoalveolar lavage fluid from patierts with lung cancer. Em J Respir Crit Care Med 1996; 154: 1706-11.

21.Colak O, Alatat O, Akyüz F, Kanbak G, Inal M. Antioxidant status in chronic obstructive pulmonary disease. Tr J of Medical Sciences 1995; 24: 305-7.

22.Moldeus P, Berggren M, Grafström R. N- Acetylcysteine protection against the toxicity of cigarette smoke coandensates in various tissues and cells in vitro. Eur J Respir Dis 1985; 66(139): 123-9.

23.Moldeus P, Cotgreave IA, Berggren M. Lung protection by a thiol-containing antiotxidant: N-acetylcysteine. Respiration 1986; 50(1): 31-42.