



THE EFFECT OF LYCOPENE SUPPLEMENTATION ON CHRONIC OBSTRUCTIVE LUNG DISEASE

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ABSTRACT

Objective: An imbalance in the oxidant-antioxidant system has been recognised as one of the first events that ultimately lead to inflammatory reactions in chronic obstructive lung disease (COPD). Lycopene, an antioxidant carotenoid, is associated with prevention of some human chronic diseases.

This study was designed to investigate the effect of lycopene on oxidant stress and pulmonary function tests (PFT) in patients with COPD.

Material and Method: Thirty clinically stable COPD patients, and 15 healthy non-smoker subjects were enrolled on the study. Fifteen COPD patients received oral placebo and 15 patients received 20 mg lycopene daily for 4 months in addition to their standardized therapy. Their baseline spirometry, plasma malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), interleukin (IL)-6, IL-1 β , and tumor necrosis factor alpha (TNF- α

levels) were measured in serum. All parameters were measured again after supplementation.

Results: The mean MDA, IL-6, IL-1 β , and TNF- α levels in the COPD group at baseline were higher than controls, while SOD and CAT levels were lower in the patients compared to controls. Four months of lycopene supplementation produced significant increase in mean SOD, and CAT levels (both $p < 0.001$), significant decrease in mean MDA, IL-6, IL-1 β , and TNF- α levels ($p = 0.001$, $p < 0.001$, $p = 0.002$, $p < 0.001$, respectively). Although no significant change in forced expiratory volume in one second (FEV₁) (% predicted) was observed after lycopene supplementation, FEV₁/FVC (%) ratio was increased ($p = 0.016$).

Conclusion: The results suggest that lycopene supplementation may have favorable effects on oxidant-antioxidant balance in patients with COPD.

Key Words: COPD, lycopene, oxidants, antioxidants
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KOAH'LI OLGULARDA LİKOPENİN ETKİLERİNİN DEĞERLENDİRİLMESİ

ÖZET

Amaç: KOAH'da inflammatuar reaksiyonlara öncülük eden önemli olaylardan biri oksidan-antioksidan sistem arasındaki dengesizliktir. Bir bioaktif karotenoid olan likopen, insanlarda bazı kronik hastalıkların gelişimini önlemektedir.

Bu çalışma likopenin oksidatif stres üzerine etkilerini araştırmak ve likopen tedavisi sonrası solunum fonksiyon testi parametrelerini değerlendirmek amacı ile planlandı.

Materyal ve Metod: Klinik olarak stabil 30 KOAH hastası ve 15 sigara içmeyen sağlıklı olgu çalışmaya alındı. KOAH'lı hastaların 15'i standart tedavilerine ek olarak 4 ay süre ile oral placebo, 15'i 20 mg/gün oral likopen tedavisi aldılar. Tüm olguların başlangıç spirometrisi, serum malonildialdehit (MDA), süperoksit dismutaz (SOD), katalaz (CAT), interlökin (IL)-6,

IL-1 β ve tümör nekrotizan faktör (TNF)- α seviyeleri ölçüldü. Likopen tedavisi sonrası tüm parametreler tekrar değerlendirildi.

Bulgular: Başlangıç değerlendirmesinde KOAH'lı hastaların serum MDA, IL-6, IL-1 β ve TNF- α seviyeleri kontrol grubundan yüksek bulunur iken, SOD ve CAT seviyeleri kontrol grubundan düşük olarak tespit edildi. Dört aylık likopen tedavisi serum SOD ve CAT değerlerinde anlamlı artışa (her ikisi için $p<0,001$), MDA, IL-6, IL-1 β ve TNF- α seviyelerinde anlamlı azalmaya (sırasıyla, $p=0,001$, $p<0,001$, $p=0,002$, $p<0,001$) neden oldu. FEV₁ değerinde anlamlı artış izlenmezken, FEV₁/FVC (%) oranındaki artış istatistiksel olarak anlamlı bulundu ($p=0,016$).

Sonuç: Bulgular likopen tedavisinin KOAH'lı hastalardaki oksidan-antioksidan denge üzerine anlamlı etkileri olabileceğini göstermektedir.

Anahtar Kelimeler: KOAH, likopen, oksidanlar, antioksidanlar *Nobel Med 2012; 8(3): 98-104*

INTRODUCTION

Chronic obstructive lung disease (COPD) is a disease state characterised by partially reversible progressive air-flow limitation associated with an inflammatory process of the lung.¹ Cell infiltration orchestrated by chemokines and other chemoattractants, the proteinase-antiproteinase system, oxidative stress and airway remodelling are central processes associated with the development of COPD.² Although the pathogenesis of COPD remains incompletely understood, the importance of oxidative stress in both lungs and systemic circulation is well established.³ Numerous reports indicate that oxidative stress is increased in COPD patients compared with either healthy subjects or smokers with similar smoking history but who have not developed COPD.^{4,5}

An imbalance in the oxidant-antioxidant system has been recognised as one of the first events that ultimately lead to inflammatory reactions in the lung.⁶ Oxidative stress also turns on the redox-sensitive transcription factors (nuclear factor- κ B [NF- κ B] and activating protein-1 [AP-1]), resulting in the production of proinflammatory cytokines and chemokines, which further aggravate inflammation and oxidative stress.⁷

Lycopene is a bioactive antioxidant carotenoid present in fruits and vegetables such as tomato, watermelon, guava and pink grapefruit. As a natural antioxidant, lycopene is associated with the prevention of some

chronic diseases including cardiovascular diseases and various cancers in the human.^{8,9} Since tobacco smoke contains more than 10^{15} oxidant molecules/puff, it is conceivable that dietary carotenoid intake may influence the progression of COPD. Despite the known antioxidant role of lycopene, we are not aware of any study that addresses the effect of lycopene on oxidative stress in COPD patients. So, this present study was designed to investigate the effect of lycopene on oxidant stress and PFT of patients with stable COPD before and after lycopene supplementation.

MATERIAL and METHOD

The Ethics Committee of Firat University Medicine Faculty reviewed and approved the protocol, and all patients signed informed consent after the nature of the study had been fully explained prior to their participation.

Patients

Thirty male nonsmoker patients with clinically stable, stage III COPD were enrolled on the study. The diagnosis of COPD was made by a pulmonary physician using certain criteria including current or ex-smoking history and largely irreversible airways obstruction with <15% improvement in baseline forced expiratory volume in one second (FEV₁) in response to inhaled β_2 agonist. The clinical condition of the patients was stable, with no acute →

Table 1: Demographic characteristics and PFT parameters of COPD patients

Parameter	COPD patients treated with lycopene (n=15)	COPD patients treated with placebo (n=15)
Age	66.88±9.87	62.93±2.68
Duration of disease (years)	10.86±11.26	11.62±2.41
Smoking (pack/year)	47.53±42.51	50.00±18.22
FEV ₁ (% predicted)	42.13±13.59	41.53±5.23
FEV ₁ /FVC (%)	47.13±9.81	51.80±11.18

PFT: pulmonary function tests, COPD: chronic obstructive lung disease

Table 2: Comparison of oxidative and inflammatory marker levels in both COPD and control groups

Parameter	COPD patients (n=30)	Healthy controls (n=15)	p value
Malondialdehyde level, mean±SD (range), nmol/mL	0.67±0.36	0.39±0.15	0.002
Superoxide dismutase level, mean±SD (range), U/mL	0.15±0.06	0.20±0.04	0.002
Catalase level, mean±SD (range), k/mL	14.58±2.85	17.37±2.60	0.004
Interleukin-6 level, mean±SD (range), pg/ml	13.58±4.62	6.23±1.73	0.000
Interleukin-1β level, mean±SD (range), pg/ml	3.87±1.79	2.84±1.55	0.042
Tumor necrosis factor-α level, mean±SD (range) pg/ml	36.20±10.22	15.80±2.83	0.000

COPD: chronic obstructive lung disease

exacerbations of COPD in the month prior to entry into the study. Patients had no other documented concurrent pulmonary or systemic disease or evidence of any upper or lower respiratory tract infection. All COPD patients were provided with a standardized therapy including inhaled salmeterol/formoterol, inhaled tiotropium bromide, oral theophylline, inhaled fluticasone/budesonide.

Thirty COPD patients were randomly divided into two groups. Fifteen of them received oral placebo and 15 patients received 20 mg lycopene daily for 4 months addition to their standardized therapy. Both lycopene and placebo capsules were supplied by Mikro-gen Istanbul, Turkey.

Control subjects

Fifteen healthy non-smoker male subjects who were matched for age from the general population participated in the study. None was receiving any medications and specifically no inhaled bronchodilators or corticosteroids. None of them had a history of systemic or lung disease.

Spirometry

The pulmonary function tests were performed using a spirometry device (Superspiro, Micromedical Limited, Rochester, England). Maximal Expiratory Flow Volume curves were obtained. Three acceptable

and at least two reproducible curves [the two highest forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁) being within 200 ml of each other] were obtained in each subject. The highest values of FEV₁ was selected for analysis.

The determination of SOD, CAT activities and MDA levels

Blood samples were centrifuged at 3000g for 10 min and sera were collected. For measurement of total (Cu-Zn and Mn) SOD (EC 1.15.1.1) activity levels, the method of Sun et al were used.¹⁰ This method is based on reduction of superoxide, which is produced by the xanthine oxidase enzyme system, by nitroblue tetrazolium. A unit of superoxide dismutase (SOD) was determined as the amount that decreases nitroblue tetrazolium reduction by 50%. Results were expressed as nmol/mL. Catalase (CAT) (EC 1.11.1.6) activity was determined by the method of Aebi.¹¹ The principle of the assay is based on the determination of the rate constant *k* (dimension: s⁻¹) of the hydrogen peroxide decomposition. By measuring the absorbance changes per minute, the rate constant of the enzyme was determined. Activities were expressed as *k* /mL. Levels of plasma malonyldialdehyde (MDA) were measured by the thiobarbituric acid (TBA) method, which was modified from methods of Satoh and Yagi.^{12,13} Peroxidation was measured as the production of MDA, which in combination with TBA forms a pink chromogen compound whose absorbance at 532 nm was measured. MDA results were expressed as nmol⁻¹ ml.

The determination of IL-6, IL-1β and TNF-α levels

Serum TNF-α concentration (pg/ml) was measured with a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) using a human TNF-α kit (BioSource International Inc, California, USA). Serum IL-6 and IL-1β concentrations (pg/ml) were also measured with ELISA kits (Biosource International Inc, California, USA).

The measurement of lycopene level

Lycopene level was measured as described previously with minor modifications using the fully automatic HPLC (Shimadzu, Kyoto, Japan), consisting of a pump (LC-20AD), a UV-visible detector (SPD-20A), an inertsil ODS-3 C18 column (250 x 4.6 mm, 5 m), a column oven (CTO-10ASVP), an autosampler (SIL-20A), a degasser unit (DGU-20A5), and a computer system with LC solution Software (Shimadzu, Kyoto, Japan).¹⁴ Briefly, serum samples were combined with a KOH/ethanol solution (1:5) containing 0.1% BHT. Samples were then placed on ice, and deionized water →

was added. Serum carotenoids were extracted four times with addition of hexane. Then, hexane extracts were evaporated under nitrogen in dark. The residues were dissolved in 100 μ L of mobil phase. All extracts were kept on ice and in subdued light. The HPLC analysis was performed on a C18- ODS-3 column with ethanol: acetonitrile: dichloromethane: water (7:7:20:0.16) containing 20 mmol/L ammonium acetate, flow rate 1 mL min⁻¹ and detection at 470 nm.¹⁵

Statistical Analysis

Data were analyzed using the statistical package for the social sciences (SPSS) v12.0 software statistical program. Results were given as group means \pm standard deviations (SD). A p value of <0.05 was considered statistically significant. Comparisons between groups were performed using the Mann-Whitney Rank Sum test. Paired t-tests were used to compare the parameters of COPD group before and after lycopene supplementation.

RESULTS

There was no difference between COPD patients and controls in age and sex distribution. Also no significant difference was observed between COPD groups in demographic characteristics and PFT parameters. The demographic characteristics and PFT parameters of all COPD patients are shown on Table 1.

Serum MDA, IL-6, IL-1 β , and TNF- α levels of COPD patients were significantly higher, serum SOD, and CAT levels were significantly lower than control group (for all, p<0.05). The oxidative and inflammatory marker levels of both COPD and control groups are shown on Table 2. There was no relation between oxidative markers and disease stage of COPD. Four months of lycopene supplementation was associated with a significant increase in mean SOD, and CAT levels (p<0.001), a significant decrease in mean MDA, IL-6, IL-1 β , and TNF- α levels (for all, p<0.05). When PFT parameters evaluated, we observed an increase in FEV₁ (% predicted), but it did not reach statistically significance. A significant increase was seen in FEV₁/FVC (%) parameter after lycopene supplementation (p=0.016) (Table 3).

When we evaluated the levels of oxidant-antioxidant, inflammatory, and pulmonary function parameters in placebo treated group, we saw no difference between pre and post treatment levels (Table 4).

DISCUSSION

Although the precise mechanisms of the pathogenesis

Table 3: Effects of lycopene supplementation (20 mg/day) for 4 months on oxidant-antioxidant, inflammatory, and pulmonary function parameters

	Baseline values	After lycopene supplementation	p value
Malondialdehyde level, mean \pm SD (range), nmol/mL	0.89 \pm 0.40	0.47 \pm 0.19	0.001
Superoxide dismutase level, mean \pm SD (range), U/mL	0.16 \pm 0.8	2.53 \pm 0.83	0.000
Catalase level, mean \pm SD (range), k/mL	15.07 \pm 3.57	56.18 \pm 17.08	0.000
Interleukin-6 level, mean \pm SD (range), pg/ml	15.20 \pm 5.68	7.29 \pm 5.83	0.000
Interleukin-1 β level, mean \pm SD (range), pg/ml	3.88 \pm 1.94	1.96 \pm 0.38	0.002
Tumor necrosis factor- α level, mean \pm SD (range) pg/ml	39.60 \pm 12.43	25.95 \pm 5.59	0.000
Lycopene level, mean \pm SD (range), mg/L	0.43 \pm 0.13	0.54 \pm 0.14	0.047
FEV ₁ (% predicted)	42.13 \pm 13.59	43.53 \pm 14.54	0.67
FEV ₁ /FVC (%)	47.13 \pm 9.81	56.13 \pm 14.36	0.016

Table 4: Levels of oxidant-antioxidant, inflammatory, and pulmonary function parameters in placebo treated group

	Baseline values	After placebo	p value
Malondialdehyde level, mean \pm SD (range), nmol/mL	0.46 \pm 0.14	0.51 \pm 0.44	0.59
Superoxide dismutase level, mean \pm SD (range), U/mL	0.15 \pm 0.22	0.15 \pm 0.12	0.62
Catalase level, mean \pm SD (range), k/mL	14.09 \pm 1.88	14.69 \pm 0.47	0.29
Interleukin-6 level, mean \pm SD (range), pg/ml	11.97 \pm 2.53	11.76 \pm 1.51	0.60
Interleukin-1 β level, mean \pm SD (range), pg/ml	3.86 \pm 1.70	3.59 \pm 0.92	0.42
Tumor necrosis factor- α level, mean \pm SD (range) pg/ml	32.80 \pm 6.09	33.40 \pm 5.72	0.52
FEV ₁ (% predicted)	41.53 \pm 5.23	42.40 \pm 2.72	0.59
FEV ₁ /FVC (%)	51.80 \pm 11.18	51.33 \pm 5.24	0.88

of COPD have not been fully elucidated, it is thought that the inflammatory responses of the lungs induced by noxious gases and particles, such as oxidants and xenobiotics, play a major role in the onset and progression of the disease. An impairment of protective mechanisms of the lungs against such harmful molecules would also contribute to the disease process.

Oxidative stress and imbalance in the host defense mechanisms appear to be among the causes of COPD. Reactive oxygen/nitrogen species have been suggested to be involved in the pathophysiology of COPD through several studies analyzing airway samples such as induced sputum.^{16,17} It has been reported that antioxidant enzyme activities are altered in COPD. The results of studies of antioxidant enzyme activities in smokers have been controversial. The activities of SOD and catalase have been reported to be increased in the alveolar macrophages from cigarette smokers and smoke-exposed hamsters.¹⁸ In one study, the mRNA expression for catalase was decreased in the peripheral lungs from COPD subjects.¹⁹ Similarly, Matskevich et al. reported that catalase activity is decreased in circulating red blood cells in patients with COPD, while the level of catalase is often increased in those from smokers, probably due to upregulation as an adaptive response against oxidative stress by cigarette smoking.^{20,21} Not only in COPD patients, but also \rightarrow

in smokers it is shown that the catalase activity in erythrocytes decreased compared with non-smokers, and was slowly increased in smokers after smoking cessation.²² Recently, Ning and colleagues have reported the decreased gene expression for catalase in surgically obtained lung tissue from patients with COPD, using a serial analysis of gene expression.²³ There are conflicting results about SOD activity in COPD patients. In one study, an increase was found in SOD activities during exacerbation when compared with stable COPD patients.²⁴ Contrastly, Daga et al showed a reduction in erythrocyte SOD activity in COPD patients.²⁵ A decreased activity of SOD was also found in alveolar macrophages of elderly smokers when compared with nonsmokers.²⁶ However, in Mak et al. study, they found no differences in erythrocyte SOD activity but elevated erythrocyte catalase activity in smokers with COPD when compared to healthy smokers matched for age and pack-years smoked.²⁷ In another study, no differences were observed in erythrocyte SOD and catalase activities between patients with moderate and severe COPD.²⁸ We found that plasma levels of SOD and CAT decreased in COPD group when compared to healthy controls. This may point towards the fact that increased production of free radicals in COPD patients leads to increased consumption of antioxidant enzymes.

Inflammatory mediators play a crucial role in chronic inflammatory processes and appear to determine the nature of the inflammatory response by directing the selective recruitment and activation of inflammatory cells and their perpetuation within the lungs. The cellular inflammatory response in COPD is dominated by neutrophils, macrophages and CD8-positive T lymphocytes in the lungs.²⁹ Direct or indirect oxidant stress to the airway epithelium and alveolar macrophages may generate cytokines, such as TNF- α , which in turn can activate airway epithelial cells to induce proinflammatory genes, such as TNF- α , IL-8, IL-1, inducible nitric oxide synthase (iNOS), cyclooxygenase-2, intracellular adhesion molecule-1, IL-6, Macrophage inflammatory protein 1 alpha (MIP-1a), Granulocyte/ macrophage colony stimulating factor (GM-CSF), stress response genes [heat shock protein (hsp)-27, 70, 90, heme oxygenase-1 (HO-1)] and antioxidant enzymes (glutamate cysteine ligase, manganese superoxide dismutase and thioredoxin).³⁰ Our results are consistent with these reports suggesting the involvement of these cytokines in the disease process of COPD.

MDA, an end product of lipid peroxidation, is used as a marker of oxidative stress.³¹ There have been many studies showed that MDA levels increased in COPD patients when compared to healthy controls.^{25,32,33} In

contrast to these findings, Karadag et al. showed similar concentrations of MDA in COPD and control group.³⁴ They suggested that serum MDA concentration may be normal in clinically stable patients. In addition, their COPD patients enrolled in this study had not smoked for at least 3 months and MDA is known to be closely related to smoking. Our study also shows increased MDA concentration in plasma of patients with stable COPD, providing indirect evidence for increased production of reactive oxygen species in these patients and hence more lipid peroxidation.

Since around the turn of the last century, a large amount of data has appeared on the importance of diet, especially of the antioxidants and micronutrients such as of vitamin C and E in the prevention and management of COPD and asthma.³⁵ Analyzing the data from the Third National Health and Nutritional Examination Survey (NHANES III) on the US population in 1988-1994 (n=18162 subjects of age 17 year or above), the authors reported a better lung function with higher levels of antioxidant nutrients.³⁶ Findings of these epidemiological observations demonstrating benefits with dietary supplements have therefore raised the hope of managing COPD with an additional approach to currently available therapy. Following vitamins E and C supplementation, some of the studies have reported a decreased lipid peroxidation in smokers or an improvement in the DNA resistance to oxidative challenge in COPD.^{37,38} But there is paucity of data on clinical improvement. Daga and colleagues from Delhi supplemented COPD therapy in a group of 30 patients with vitamin E administration for 12 weeks.²⁵ They failed to demonstrate any improvement in the spirometric measurements although the levels of plasma MDA were shown to decrease. In another study, the authors report an augmentation of antioxidant defenses but no clinical benefit following eight weeks of exogenous vitamin E supplementation in ten COPD patients.³⁹ Habib et al. found no effect of 400 IU vitamin E supplementation twice a day for 3 weeks on exhaled ethane levels (a marker of oxidative stress) in smokers.⁴⁰ Brown et al. found significant reductions in plasma lipid peroxides and conjugated dienes after 10 weeks of 280 mg vitamin E supplementation once daily in smokers.³⁷ The disagreement in literature may relate to the doses and duration of supplementation and whether the subjects had COPD or not and the different methods used by different researchers.

Lycopene is another important antioxidant used in studies. Arab et al. reported that greater levels of lycopene and other carotenoids in the lung may provide an additional level of protection against oxidative and ozone induced damage.⁴¹ Concentrations →

of carotenoids in addition to the other antioxidant vitamins in the lung epithelial lining and lining fluids can provide protection against oxidative damage. Case-control and cohort studies have examined lycopene-rich diets and lung cancer.^{42,43} Cohort studies do not show an association between lycopene and/or tomatoes and risk of lung cancer, whereas case-control studies consistently show a protective effect for the highest categories of consumption. The authors comment that these results may be due to the decreased attention to quantitative dietary assessment in cohort studies, measurement error in case-control studies, or the differences in bioavailability assessment between studies. In contrast, a recent study about long-term use of beta-carotene, retinol, lycopene, and lutein supplements and lung cancer risk showed an increased cancer risk in these individuals.⁴⁴

In a study, investigating the effect of lycopene supplementation on biomarkers of oxidative stress, lycopene was shown to decrease DNA oxidative damage and urinary 8-hydroxy deoxyguanosine at the high dose.⁴⁵ An experimental study in a rat model of acute lung injury showed that lycopene might attenuate lipopolysaccharide-induced impairment of lungs and improve acute lung injury (ALI)

prognosis by increasing the phagocytic function of PAMs significantly and restraining the secretion of TNF- α and IL-8.⁴⁶ Lee et al also showed the beneficial effect of lycopene in a murine model of asthma.⁴⁷ They showed a significant inhibition of the infiltration of inflammatory immunocytes into the bronchoalveolar lavage and a reduction in the levels of GATA-3 mRNA and IL-4 expression in ovalbumin (OVA)-challenged mice by lycopene treatment. Kasagi et al generated a mouse model of emphysema by tobacco smoke inhalation, and they evaluated the lycopene effect on the development of emphysema.⁴⁸ They confirmed that lycopene concomitantly given with chronic tobacco smoke exposure completely protected mice from smoke-induced emphysema. We also showed the beneficial effect of lycopene on inflammatory markers and oxidant-antioxidant balance in patients with COPD.

In conclusion, we think that lycopene supplementation may have favorable effects on oxidant-antioxidant balance in patients with COPD. However, the lack of a significant effect on FEV₁ (% predicted) could be due to its short term use in this clinical setting. A large population-based cross-sectional study of longer duration could better reflect long-term effect of this antioxidant on pulmonary function in COPD patients.



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