Effects of exogenous vitamin E supplementation on the levels of oxidants and antioxidants in chronic obstructive pulmonary disease

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Oxidative stress has been recognized as a central feature of smoke induced chronic obstructive pulmonary disease (COPD). Imbalance between oxidant and antioxidant enzymes is also an established fact in these patients. But studies in regard to stable COPD patients and effect of vitamin E supplementation are lacking. Thirty patients with COPD were included in the study. Their baseline clinical examination, spirometry, plasma malondialdehyde (MDA), alpha-tocopherol and red blood cell superoxide dismutase (SOD) levels were measured. Twenty healthy non-smokers who were matched for age and sex served as controls. All the above parameters were repeated after 12 weeks of supplementation with 400 IU of vitamin E daily. The mean malondialdehyde levels in the patients at baseline were higher than controls (5·91 ± 1·23 nmol/ml vs 4·55 ± 1·51 nmol/ml, $P = 0{\cdot}001$), so also was plasma alpha-tocopherol levels ($P < 0{\cdot}001$), while SOD levels were lower in the patients compared to controls (1692 ± 259 units g/Hb vs 2451 ± 131 units g/Hb, $P < 0{\cdot}001$). Exogenous vitamin E (400 IU per day) supplementation did not bring about any significant change in plasma alpha-tocopherol and SOD levels. The Pearson's co-efficient of correlation between the levels of MDA, vitamin E, SOD; and spirometric measurements were not significant either on day 1 or after 12 weeks of vitamin E supplementation. The present study shows that initially the plasma lipid peroxide (MDA) levels are high and antioxidants (alpha-tocopherol and SOD) are low in patients with COPD. Exogenous supplementation with vitamin E does not have any significant effect on the spirometric measurements though it brings down the levels of MDA showing attenuation of further damage. However, inclusion of larger number of patients and supplementation with vitamin E for longer periods may throw more light on free radical injury and protective effects of antioxidants.

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1. Introduction

Chronic obstructive pulmonary disease (COPD) – a chronic slowly progressive disorder – is characterized by airflow obstruction. It is defined as forced expiratory volume in first second (FEV1) less than 80% of predicted and FEV_1/forced vital capacity (FVC) ratio less than 70% of predicted, that does not change markedly over several months of observation (American Thoracic Society 1987). The World Bank estimates that COPD is responsible for greater than 29 million disability-adjusted life years and one million years of life lost per annum around the world. Smoking accounts for 90% of cases of COPD, but only 15% of smokers develop clinically symptomatic COPD.

Oxidative stress has been implicated in the pathogenesis of tobacco-smoke-induced chronic obstructive pulmonary disease (Brigham 1986). Lannan *et al* (1994) showed that reactive oxygen species in tobacco smoke may cause

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Abbreviations used: COPD, Chronic obstructive pulmonary disease; FEV, forced expiratory volume; FVC, forced vital capacity; MDA, malandialdehyde; PEFR, peak expiratory flow rate; SOD, superoxide dismutase.

damage to human alveolar epithelial cells by lipid peroxidation of cell membranes, decreased epithelial cell adherence and increased detachment and lysis of epithelial cells. Reduced levels of major plasma antioxidants (vitamin E, ascorbic acid) have been demonstrated in bronchoalveolar lavage fluid of smokers compared with those in nonsmokers (Pacht *et al* 1986). Enhanced activities of antioxidant enzymes, superoxide dismutase (SOD) and catalase have also been demonstrated in alveolar macrophages from young smokers (McCusker and Hoidal 1990). Based on accumulating evidence that oxidants or free radicals play an important role in the pathogenesis of COPD, enhancing the pulmonary antioxidant capacity, may be of potential therapeutic benefit in this condition. The only definitive method for measuring free radical activity in body tissues is by electronic spin resonance which is technically difficult. A practical method for measurement of free radical activity in biological fluids is by estimating the levels of malondialdehyde (MDA), ethane and propane which are lipid peroxidation products formed by free radical attack on polyunsaturated fatty acids in the cell membrane.

There are very few studies done on COPD patients correlating the disease with oxidant and antioxidant levels. In this study we compared the oxidation product (MDA) and antioxidant SOD levels in COPD and healthy nonsmokers. Further, we tested the effect of vitamin E supplementation at 400 IU per day for 12 weeks on MDA and SOD levels and pulmonary function tests (FEV_1, FEV_1/FVC and peak expiratory flow rate, PEFR).

2. Materials and methods

This was a randomized, controlled, double blind prospective clinical study. This study was undertaken in the Department of Medicine and Biochemistry, Lok Nayak Hospital, Maulana Azad Medical College, New Delhi, India. Thirty patients with chronic obstructive lung disease with $FEV_1 < 80\%$, $FEV_1/FVC < 70\%$ and age > 35 years from the pulmonary outpatient department of Lok Nayak hospital were included in the study. Twenty four patients (80%) were male and six (20%) were female. The study was approved by the Ethics Committee of the Lok Nayak Hospital and all patients gave written informed consent. Patients under 35 years or on any vitamin supplementation for at least 4 weeks or with systemic disease or showing $FEV_1 < 80\%$ with reversibility > 15% or in acute exacerbation were excluded from the study. The patients were evaluated by thorough medical history and complete physical examination. Particulars like name, age, sex, address, duration of smoking and chief complaints were noted. Routine hematological and biochemical investigations were carried out for each patient. Twenty healthy nonsmokers matched for age and sex were selected as controls.

Spirometry (Master Screen Diffusion, JAEGER, Germany) was performed on day 1 at the Respiratory Laboratory, Pulmonary Medicine Division of Lok Nayak Hospital. The calculations were performed using Udwadia's formula (Udwadia *et al* 1987). Baseline MDA levels were estimated using thiobarbituric acid (TBA) reaction and Beckman spectrophotometer following the method described by Asakawa and Matsushita (1980). Erythrocyte SOD levels were estimated based on the inhibition of pyrogallol autoxidation caused by SOD as described by Markland and Markland (1974) and modified by Nandi and Chatterji (1988). Plasma alpha-tocopherol was estimated by Hansen and Warwick method (Hansen and Warwick 1969) by plotting relative fluorometer units vs concentration of the tocopherol standards on a standard curve. Fifteen patients were randomly chosen to receive vitamin E at 400 IU/day (given as Cap Evion) for 12 weeks. After 12 weeks, spirometry and measurement of MDA, SOD and alpha-tocopherol was repeated. The standard supportive treatment for COPD was continued in all the patients for 12 weeks. The outcome variables were: FEV_1, FEV_1/FVC, PEFR, MDA levels, SOD levels and alpha-tocopherol levels.

The statistical analyses were performed using *t* test, and *P* values < 0·05 were interpreted as statistically significant. Pearson's coefficient was used to determine correlation between MDA levels, alpha-tocopherol levels, SOD and spirometric measurements.

3. Results

The mean MDA level in patients ($n = 30$) was 29·7% higher than controls ($n = 20$) at baseline (5·91 ± 1·23 nmol/ml vs 4·55 ± 1·57 nmol/ml, $P = 0·001$). Mean plasma alpha-tocopherol level in patients (7·09 ± 2·14 μg/ml) was 28·67% lower than controls (9·94 ± 2·01 μg/ml, $P < 0·001$).

Mean SOD level in patients was 30·9% lower than controls (1692 ± 259 units/gmHb vs 2451 ± 131 units/gHb, $P < 0·001$). Thus, at baseline, smokers with COPD had higher MDA levels but lower antioxidant (SOD and tocopherol) levels than healthy controls (table 1).

Twelve weeks of vitamin E supplementation produced 42·8% reduction in mean MDA level (3·37 ± 0·81 nmol/ml vs 5·91 ± 1·43 nmol/ml, $P < 0·001$). However, no significant change in mean plasma alpha-tocopherol level (7·71 ± 2·62 μg/ml vs 7·22 ± 2·24 μg/ml, $P = 0·623$) and mean SOD level (1693 ± 179 U/gHb vs 1622 ± 253 U/gHb, $P = 0·263$) was observed after 12 weeks of vitamin E supplementation. The Pearson's coefficients of correlation between MDA levels, tocopherol, SOD and spirometric measurements were not significant on either day 1

or after 12 weeks of vitamin E supplementation. No significant improvement in FEV_1, FEV_1/FVC and PEFR was observed after 12 weeks of vitamin E supplementation (table 2).

4. Discussion

The mean lipid peroxide (MDA) level for the healthy controls was 4·55 ± 1·57 nmol/ml as estimated by Asakawa and Matsushita (1980) method. Yagi (1987) has reported a mean value of 3·94 ± 0·70 nmol/ml. The differences in these values may be attributable to the differences in the methods. It could also be due to the fact that plasma lipid peroxidation has a wide range and varies with the age and diet of the individual (Yagi 1987).

This study 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 (29·7% higher MDA levels in patients than controls, $P = 0·001$). Our results are consistent with those by Dekhuijzen *et al* (1986) who measured hydrogen peroxide instead of MDA as a marker of lipid peroxidation and showed increased exhalation in patients with stable COPD. They also showed further increase in patients with acute exacerbation. Similarly, increased TBA MDA levels and low trolox equivalent antioxidant capacity (TEAC) levels have been demonstrated in acute exacerbations of COPD (Rahman *et al* 1996).

In this study, vitamin E supplementation for 12 weeks produced 42·8% reduction in lipid peroxidation levels (MDA), ($P < 0·001$). In a similar study, vitamin E supplementation (300 IU twice a day) in stable smokers was studied (Habib *et al* 1999). They observed that vitamin E supplementation did not reduce ethane (a marker of lipid peroxidation) significantly. Habib *et al* (1999) studied the levels in healthy smokers, but the duration of therapy was only for 3 weeks which might be inadequate to produce any significant effect. We administered vitamin E in dose of 400 IU daily for 12 weeks. Our study demonstrates

Table 1. Comparison of mean plasma MDA level, plasma tocopherol levels and SOD levels in smoker COPD patients and healthy controls at baseline.

Parameter	COPD patients (smokers) ($n = 30$)	Healthy controls ($n = 20$)	*P* value
Malondialdehyde levels, mean ± SD (range), nmol/ml	5·91 ± 1·23 (2·9–7·8)	4·55 ± 1·57 (3·9–8·4)	0·001*
Alpha-Tocopherol levels, mean ± SD (range), μg/ml	7·09 ± 2·14 (3–11·93)	9·94 ± 2·01 (6·8–13·56)	< 0·001*
Erythrocyte SOD levels, mean ± SD (range), units/gmHb	1692 ± 259 (1322–2325)	2451 ± 131 (2278–2650)	< 0·001*

*Significant *P* values.

Table 2. Effect of vitamin E supplementation (400 IU/day) for 12 weeks on MDA levels, SOD levels, alpha-tocopherol levels, forced expiratory volume in first second (FEV_1), FEV_1/FVC and peak expiratory flow rate (PEFR) in smokers with COPD ($n = 15$).

Parameter	Baseline values (day 1)	After vitamin E supplementation (12 weeks)	*P* value
Malondialdehyde levels, mean ± SD (range), nmol/ml	5·91 ± 1·23 (2·9–7·8)	3·37 ± 0·81 (1·9–5·1)	< 0·001*
SOD levels, mean ± SD (range), units/gmHb	1622 ± 253 (1322–2325)	1693 ± 179 (14·20–1960)	$0·263^{ns}$
Alpha-tocopherol levels, mean ± SD (range), μg/ml	7·22 ± 2·24 (3·94–11·03)	7·71 ± 2·62 (1·30–11·08)	$0·623^{ns}$
FEV_1, mean + SD (range), L/min	1·27 ± 0·22 (0·92–1·69)	1·27 ± 0·23 (0·88–1·65)	$0·801^{ns}$
FEV_1/FVC, mean ± SD (range), percent	62·07 ± 8·66 (47·2–73·9)	62·38 ± 11·42 (37·40–76·79)	$0·845^{ns}$
PEFR, mean + SD (range), L/min	3·64 ± 0·62 (2·53–4·12)	3·72 ± 0·72 (2·45–5·2)	$0·319^{ns}$

*Significant *P* value; ns, non-significant *P* value.

that vitamin E, under our protocol, is an effective antioxidant, as it reduced the level of plasma MDA by inhibiting lipid peroxidation caused by free radicals.

The mean plasma alpha-tocopherol level was 28·7% lower in smokers than controls ($P < 0{\cdot}001$) in our study. Similarly, Pacht *et al* (1986) reported lower bronchoalveolar lavage fluid level of alpha-tocopherol in smokers compared with non-smokers. It is a well known fact that COPD develops in only 15% of chronic smokers. The fact that smokers have lower tocopherol levels, and thus are more prone to free radical injury, may be used in preventing the development of COPD in future by supplementation of vitamin E. It may also be used to prevent the rate of decrease in FEV_1/year by preventing further damage to the lung tissue.

We found no significant increase in plasma tocopherol levels in COPD patients after 12 weeks of vitamin E supplementation ($P = 0{\cdot}623$). However, another study showed increase in tocopherol levels in smokers after vitamin E supplementation at 1000 mg/day for 14 days (Guthrie *et al* 1995). Pacht *et al* (1986) demonstrated increase in alpha-tocopherol concentration in broncoalveolar lavage fluid after vitamin E supplementation at 2400 IU/day. Their different results can be explained on the basis of using higher dosages of vitamin E.

The mean plasma SOD level in controls was 2451 ± 131 units/gHb with the range of 2278–2650 units/gHb which is in the normal range. Winterbourne *et al* (1974) showed SOD levels of 2400–3900 units/gHb with a mean of 2900 units/gHb in healthy adults. SOD levels were 30·9% lower ($P < 0{\cdot}001$) in smokers compared to controls in our study. In a similar study, Kondo *et al* (1994) found increase in the level of oxygen radical species and decrease in the levels of SOD in alveolar macrophages from elderly chronic smokers. This may point towards the fact that increased production of free radicals in COPD patients leads to increased consumption of SOD, an antioxidant enzyme.

However, vitamin E supplementation in our study caused no significant increase in SOD levels, thus indicating that vitamin E which is a nonenzymatic antioxidant has no effect on the levels of SOD. Probably the two act independent of each other in protecting COPD patients from free radical damage. No similar study has been reported in literature.

We observed no significant improvement in pulmonary function parameters (FEV_1, FEV_1/FVC, PEFR) after vitamin E supplementation. However, another study reported no beneficial effect of alpha-tocopherol (50 mg/ day) on COPD symptoms (Rautalahati *et al* 1997). In NHANES 3, authors reported that higher levels of antioxidant nutrients were associated with better lung function (Guizhou and Patricia 2000). However, NHANES 3 data reflects antioxidant nutrient levels over a long period of time, while our study was of limited duration. A large population-based cross-sectional study of longer duration may reflect long-term effect of these antioxidants on pulmonary function in COPD patients.

In conclusion, our study shows that initially the plasma-lipid-peroxidation products (MDA levels) are high, and antioxidants (alpha-tocopherol and SOD) are low in patients with COPD. Exogenous supplementation of vitamin E does not have any significant effect on the spirometric measurements though it brings down the level of MDA suggesting attenuation of oxidative damage. The lowering of lipid peroxides may prove beneficial by preventing further damage. Larger studies with vitamin E supplementation for longer periods may throw more light on the role of free radical injury and protective effects of antioxidants in COPD.

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