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A Study of Efficacy of Antioxidant Therapy in the Management of Chronic Obstructive Pulmonary Disease

Kanani NJ*, Patel SA, Hotchandani SC and Bhatt JD

Dept. of Pharmacology, Medical College, Baroda - 390001

ABSTRACT

To study the effects of vitamin C and vitamin E therapy on oxidative stress and pulmonary function parameters in patients of COPD. The study subjects included two major groups consisting of 15 smokers without COPD and 50 smokers with COPD patients. The COPD groups of patients receiving conventional treatment were further divided into 3 groups. The first group (n=16) received vitamin C (500mg/day), the second group (n=18) received vitamin E (200mg/day) and the third group (n=16) received combination of vitamin C (500mg/day) and vitamin E (200mg/day) therapy for 3 months along with their conventional therapy. Treatment with antioxidant therapy with vitamin C and /or vitamin E produced significant improvement in values of markers of oxidative stress i.e. lowering of malondialdehyde (MDA) and elevation of reduced glutathione (GSH) without significant improvement in the pulmonary function parameters. Thus, antioxidant therapy along with conventional therapy in the management of COPD produced significant decrease in oxidative stress. However, there was no significant improvement in pulmonary function test parameters. It is concluded that antioxidant therapy produced significant reduction in the markers of oxidative stress in COPD patients. Further work is required to correlate and confirm the above observations with clinical benefits.

Keywords: Vitamin C and vitamin E, oxidative stress, COPD

Corresponding author

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) state is characterized by airflow limitation that is not fully reversible. Smoking is the main etiology factor in COPD. Cigarette smoke contains around 10^{17} oxidant molecules per puff and this, together with a large body of evidence demonstrating increased oxidative stress in smokers with and without COPD, has proposed the role of oxidant / antioxidant imbalance in the pathogenesis of this condition [1].

Lungs have an extensive, potent antioxidant defense system; present both extra and intra cellularly, which protects lung from oxidant damage [2]. Oxidative stress produces irreversible damage to DNA and various cell constituents, deficiency in antioxidants, inactivation of antiproteases and lipid peroxidation. Consequently all of these pathological changes contribute to pathogenesis of COPD [3].

Reduced levels of major plasma antioxidants (vitamin C and vitamin E) have been demonstrated in the broncholavage fluid of smokers compared with those in the nonsmokers [4].

Vitamin C reacts directly with superoxide anions, hydroxyl radicals and peroxy radicals in aqueous solutions and maintains vitamin E in reduced state [5-7]. Vitamin E, the most effective chain – breaking, membrane associated antioxidant, competes for peroxy radicals at a faster rate than polyunsaturated fatty acids, protect polyunsaturated fatty acids against oxidation [8, 9].

Based on, above clinical reports, it is likely that enhancing antioxidant capacity in lungs would prevent further complication or progression of the disease and offer potential therapeutic benefits to the patients of COPD. Therefore in the present study the effect of vitamin C and vitamin E therapy on oxidative stress and pulmonary function parameters in the patients of COPD, receiving conventional therapy was planned.

MATERIALS AND METHODS

STUDY SUBJECTS

This is an open, comparative study and the subjects with the following inclusion and exclusion criteria were selected for the study from outdoor patients attending OPD at Sir Sayajirao General Hospital Vadodara.

INCLUSION CRITERIA

1. Known case of mild and moderate COPD as per COPD diagnosing criteria [Patients with FEV_1/FVC % < 70 were included in the study and FEV_1 % predicted parameter was used to categorize the patient to mild, moderate and severe COPD as per classification of

COPD by severity[10]. Baseline values of FVC and FEV1 in adult male were 4800 ml and 3800 ml respectively while they were 3300 ml and 2800 ml respectively for adult female.

2. Male patients with age ranging 45-85 years.
3. History of smoking.
4. Patient should be otherwise healthy as confirmed by laboratory screening and baseline physical examination.
5. Healthy smokers within the same age range were also included as a control group (FEV1/FVC > 70 %)

EXCLUSION CRITERIA

1. Patients suffering from severe COPD, Diabetes Mellitus, Hypertension, Ischemic heart disease, Tuberculosis, Heart failure, Asthma, Liver disease, Cancer, Autoimmune diseases or any other systemic disease.
2. Acute exacerbation of COPD.
3. COPD patients on oral or inhaled corticosteroids.
4. Hypersensitivity to vitamin C and vitamin E.
5. Vitamin supplementation at least during the last four weeks.

Purpose of study was explained and consent was obtained from all the subjects before the study was started as per the guidelines of the institutional ethics committee.

STUDY DESIGN

Mild and moderate, known cases of COPD were selected for the study. Study subjects were allowed to continue their conventional medications (i.e. bronchodilators & anticholinergic drugs etc.). Before enrolling patients to the study, detailed medical history and physical examination were performed. Pulmonary function tests were performed using spirometry. Baseline blood investigations along with markers of oxidative stress were also measured.

Patients were then, randomized to the following study groups:

Group I (n=16) : COPD patients on conventional treatment were prescribed vitamin C 500 mg/ day for 3 months.
Group II (n=18) : COPD patients on conventional treatment were prescribed vitamin E 200 mg/ day for 3 months.
Group III (n=16) : COPD patients on conventional treatment were prescribed vitamin C 500 mg/ day and vitamin E 200 mg/ day for 3 months.

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Healthy smokers without COPD as mentioned in the inclusion criteria were selected as a control group. Study subjects had undergone routine base line investigations, markers of oxidative stress i.e. MDA and GSH and pulmonary function tests. At the end of 3 months of treatment, again medical history, physical examination, pulmonary function tests and blood investigation for markers of oxidative stress were done.

STATISTICAL ANALYSIS

All the data are expressed as mean ± standard error of mean (S.E.M). The data was analyzed statistically by using paired 't' test and post hoc one way ANOVA. $P<0.05$ was considered statistically significant.

RESULTS

DEMOGRAPHIC CHARACTERISTIC OF SUBJECTS

Control subjects and COPD patients were selected in the age range of 45-85 years. Average age of both the groups was nearly identical i.e. 69.66 years in control group and 71.24 years in COPD group. Smoking habit in control subjects and COPD patients were 33.8 and 31.46 pack years respectively (Table -1)

TABLE 1: **DEMOGRAPHIC CHARACTERISTICS OF SUBJECTS**

Characteristics	Control Subjects (n=15)	COPD Patients (n=50)
Age (years)	69.66 ±1.31	71.24±0.56
Smoking habit (Pack-years)*	33.80±1.37	31.46±2.42

* Pack years = Number of pack of cigarette smoked in one year, multiply number of years cigarette smoked. All the values are expressed as Mean ± SEM.

EFFECTS ON DIFFERENT PARAMETERS AFTER TREATMENT WITH VITAMIN C, VITAMIN E AND COMBINED TREATMENT OF VITAMIN C AND VITAMIN E FOR 3 MONTHS IN COPD PATIENTS

Treatment of COPD patients produced significant reduction in MDA values with vitamin C ($P< 0.05$) and vitamin E ($P< 0.001$) alone as well as combined treatment consisting of vitamin C and vitamin E ($P< 0.001$) for 3 months while there was significant elevation in GSH values in the group receiving combined treatment ($P< 0.001$) (Table-2). Pulmonary function tests like FEV1% predicted and FEV1/FVC % were not altered after any of the above treatments in COPD patients (Table-2).

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TABLE-2: EFFECTS ON DIFFERENT PARAMETERS AFTER TREATMENT WITH VITAMIN C, VITAMIN E AND COMBINED (VITAMIN C + VITAMIN E) TREATMENT FOR 3 MONTHS IN COPD PATIENTS

	Vitamin C (n=16)		Viamin E (n=18)		Vitamin C + Vitamin E (n=16)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
MDA (mmoles/ml)	5.56±0.27	* 4.36±0.40	5.91±0.31	** 4.11±0.28	5.43±0.21	** 2.95±0.21
GSH (Gm/Hb %)	7.21±0.28	7.44±0.29	7.05±0.26	7.19±0.23	7.11±0.30	** 10.32±0.28
FEV_1% Predicted	45.01±2.16	45.43±2.17	45.11±1.91	46.07±1.57	46.71±2.81	47.34±2.62
FEV_1/FVC %	53.45±2.28	53.55±2.38	52.50±2.05	52.57±1.43	53.06±2.73	53.16±2.52

All the values are expressed as Mean ± SEM. * P < 0.05, ** P < 0.001 as compared to value before treatment
MDA = Malondialdehyde, GSH = Reduced glutathione
FEV_1% predicted = Forced expiratory volume at 1 st second as compared to a normal person with same age, height and weight, in percentage
FEV_1/FVC % = Ratio of forced expiratory volume and forced vital capacity

COMPARISON AMONG DIFFERENT PARAMETERS OF THE COPD PATIENTS RECEIVING DIFFERENT TREATMENTS AND CONTROL GROUP AT THE END OF 3 MONTHS

Combined treatment with vitamin C and vitamin E for 3 months, produced statistically significant reduction in MDA value as compared to group receiving vitamin C (P< 0.01) and vitamin E (P< 0.05) therapy alone.. Significant elevation in the values of GSH as compared to that observed in the group receiving either vitamin C (P< 0.001) or vitamin E therapy (P< 0.001) (Table 3). Treatment with either vitamin C or vitamin E recovered the values of MDA and GSH equivalent to that observed in the control COPD patients.

Pulmonary function parameters did not show any alterations after any of the above treatments at the end of 3 months (Table 3).

CHANGES IN DIFFERENT PARAMETERS IN THE COPD PATIENTS AFTER TREATMENTS WITH DIFFERENT ANTIOXIDANT THERAPY

Combined treatments of vitamin C and vitamin E comparatively produced more significant reduction in MDA values as compared to vitamin C therapy alone (P< 0.05) and more significant elevation in the values of GSH as compared to that observed after either vitamin C (P< 0.001) or vitamin E (P< 0.001) treatment alone. However, neither of the treatments produced any significant changes in the pulmonary function parameters (Table-4).

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TABLE- 3: COMPARISON OF DIFFERENT PARAMETERS OF CONTROL GROUP WITH THE PARAMETERS OF COPD PATIENTS RECEIVING DIFFERENT TREATMENTS AT THE END OF 3 MONTHS

	MDA (mmoles/ml of serum)	GSH (Gm/Hb %)	FEV_1% predicted	FEV_1/FVC %
Control (n=15)	2.25±0.26	11.28±0.26	75.6±0.89	82.46±1.15
Vitamin C (n=16)	* 4.36±0.40	* 7.44±0.29	* 45.43±2.18	* 53.55±2.38
Vitamin E (n=18)	* 4.11±0.28	* 7.19±0.23	* 46.07±1.57	* 52.57±1.43
Vitamin C+ Vitamin E (n=16)	ac 2.95±0.21	bd 10.32±0.28	* 47.34±2.62	* 53.16±2.52
F(d,f) P	10.89(3,16) <0.0001	59.69(3,16) <0.0001	54.58(3,16) <0.0001	53.549(3,16) <0.0001

All the values are expressed as Mean ± SEM.
* P< 0.001 between control group vs after treatment group.
a P< 0.01; b P< 0.001, vitamin C vs vitamin C + vitamin E group.
c P< 0.05; d P< 0.001, vitamin E vs vitamin C + vitamin E group.

TABLE- 4: CHANGES IN DIFFERENT PARAMETERS IN THE COPD PATIENTS AFTER TREATMENTS WITH ANTIOXIDANT THERAPY

	Reduction in MDA (mmoles/ml)	Elevation in GSH (Gm/Hb %)	Elevation in FEV_1% predicted	Elevation in FEV_1/FVC %
Vitamin C (n=16)	1.25±0.34	0.22±0.12	0.41±0.29	0.16±0.26
Vitamin E (n=18)	1.80±0.30	0.18±0.08	0.95±0.61	0.10±0.25
Vitamin C+Vitamin E (n=16)	a 2.60±0.29	b c 3.08±0.25	0.58±0.52	0.10±0.29
F(d,f) P	4.596(2,47) 0.015	103.98(2,47) <0.0001	0.309(2,47) 0.7356	0.01657(2,47) 0.9836

All the values are expressed as Mean ± SEM.
a P<0.05 ; b P<0.001, vitamin C vs vitamin C + vitamin E group.
c P<0.001, vitamin E vs vitamin C + vitamin E group.

DISCUSSION

COPD is characterized by progressive and irreversible airway obstruction. Although the mechanism of airflow obstruction is not clearly understood, it is suggested that oxidant / antioxidant imbalance plays an important role in the pathogenesis of COPD [11].

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. Plasma levels of lipid

peroxide products measured as TBA (Thiobarbituric acid) – MDA derivatives, were higher in COPD patients compared to normal subjects [12, 13].

Reduced glutathione (GSH) is a well-known extra cellular antioxidant that protects tissues from the effects of oxidative stress [14]. Calikoglu et al. [15] has reported significantly lower levels of GSH in patients with COPD with negative correlation between MDA and GSH levels of erythrocyte in the patients with COPD.

It is believed that ascorbate produce 9% of plasma antioxidant capacity [16]. Vitamin C is an important antioxidant that directly neutralizes free radicals[17], suppresses macrophage secretion of super oxide anion[18] and is part of the glutathione peroxide pathway for repairing oxidative damage to the lipid membrane[19]. Calikoglu et al. [22] and Birgul et al. [20] have reported lower baseline FEV_1 % predicted values along with high values of MDA.

Pryor et al. [21] has shown that vitamin C can protect human α-1 protease inhibitor from inactivation from cigarette smoke as well as scavenging free radicals generated by activated neutrophils. The negative relationship between vitamin C and MDA may be due to depletion of vitamin C when oxidative burden is increased [22]. Our study has shown reduction in MDA levels after vitamin C supplementation for 12 weeks, however, GSH and pulmonary function parameters did not show any significant alteration.

Vitamin E is a lipid soluble molecule that functions as a chain – breaking antioxidant. Petruzzelli et al. [23] has demonstrated that serum vitamin C, vitamin E, beta – carotene and selenium were depleted in chronic smokers.

In addition to direct antioxidant effect, vitamin C reacts with aqueous peroxy radicals and indirectly stores the antioxidant properties in fat soluble vitamin E [24]. Also the tocopherol radical reconverts to alpha – tocopherol by reaction with vitamin C [25]. Bao – Khanh et al. [26] has reported that antioxidant vitamin supplementation (vitamin E, vitamin C and beta carotene) resulted in attenuation of lipid peroxidation which is associated with preserved lung function. Our study has also demonstrated more significant elevation of GSH and significant reduction of MDA levels in plasma of COPD patients after combined therapy with vitamin C and vitamin E.

Antioxidant therapy, as an adjunct to diet is effective on oxidant / antioxidant balance but has no demonstrable effects on pulmonary function tests and alveolar capillary permeability in COPD patients [27]. Results of our study did not demonstrate any positive correlation between antioxidant therapy (vitamin C and/ vitamin E) and pulmonary function test parameters. This could be explained on the basis that short term therapy (i.e. 3 months) was employed in our study as compared to long term therapy used in the above studies. Thus, antioxidant therapy may produce protective action by decreasing oxidative stress in COPD patients.

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