Evaluation of anti-oxidant and oxidative stress parameters in chronic obstructive pulmonary disease

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Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) is an important lung disorder. Tobacco smoking accounts for 80% of patients of COPD cases and the remaining 20% are due to exposure to environmental tobacco smoke, occupational dusts and chemicals, and indoor air pollution from biomass fuel used for cooking and heating in poorly ventilated buildings. The oxidative stress seen with COPD patients is due to the oxidants present in cigarette smoke and from increased amounts of reactive oxygen species released from leucocytes, from both in air spaces and blood.

Materials and Methods: This was prospective, case-control study conducted at a tertiary care teaching hospital (medicine department) from April 2015 to August 2015. After obtaining written informed consent a total of 84 participants were explained about the study. The anti-oxidant parameters i.e. Vitamin C and E, superoxide dismutase, whole blood reduced glutathione and oxidative stress markers i.e. malondialdehyde, serum nitric oxide were measured both in COPD patients and controls. The study was approved by institutional ethics committee. The results are expressed as mean and standard deviation of the parameters evaluated. The unpaired 't' test wass used for comparing different biochemical parameters between cases and controls. The p value of < 0.05 was considered as statistically significant.

Results & Discussion: There was no statistically significant difference in the demographic data between the cases and controls. Maximum patients were in stage 3 COPD. Tobacco smoking is an important cause for development of COPD by formation of reactive oxygen species and other free radicals There was statistically significant difference (p<0.001) in anti-oxidant levels (Vitamin C and E, superoxide dismutase, Whole blood reduced glutathione) and oxidative stress markers (malondialdehyde, serum nitric oxide) and between cases and controls.

Conclusion: The results of the present study indicate a disturbance in oxidant/antioxidant status in COPD patients. The periodic measurements of oxidative stress markers and antioxidant status in COPD patients might be useful in prognosis of the disease. These results may contribute for the development of novel therapeutic interventions.

Keywords: Anti-oxidant, Chronic obstructive pulmonary disease (COPD), Free radicals, Oxidative stress.

Introduction

Chronic obstructive pulmonary disease (COPD) is defined as "A disease state characterized by the presence of air flow obstruction due to chronic bronchitis or emphysema; the airflow obstruction is generally progressive, may be accompanied by airway hyper-reactivity, and may be partially reversible". 80% of patients of COPD cases are due to smoking tobacco, and 20% have a combination of exposures to environmental tobacco smoke, occupational dusts and chemicals, and indoor air pollution from biomass fuel used for cooking and heating in poorly ventilated buildings.[1]

COPD includes two conditions, chronic bronchitis, a condition of large-airway inflammation and remodeling, and emphysema, disease of the distal airways and lung parenchyma that manifests as loss of surface area for gas exchange.[2] The oxidative stress seen with COPD patients are from the oxidants present in tobacco smoke and from the increased levels of reactive oxygen species released from leucocytes, from both in air spaces and blood.[3] Oxidative stress leads to increased concentration of free radicals which can cause damage to the lipids, proteins and nucleic acids (DNA). Polyunsaturated fatty acids (PUFA) present in the cell membrane are also one of the important target of oxidants.[4]

Although there are studies conducted on anti-oxidant levels in COPD patients, the results are not consistent with oxidant parameters and antioxidant levels in the pathogenesis of COPD.[5,6] Hence the present study was with an objective to know the levels of oxidative stress

markers (malondialdehyde, serum nitric oxide) and anti-oxidants (Vitamin C and E, superoxide dismutase, Whole blood reduced glutathione) in patients with COPD.

Material and Methods

This was prospective, case-control study conducted at a tertiary care teaching hospital (medicine department) from April 2015 to August 2015. The objectives of the study were explained to the participants and informed consent was taken. The study was approved by institutional ethics committee. A total of 84 subjects (42 cases and 42 controls) were included in the study. A COPD case was defined according to the criteria of the Global Initiative for Chronic Obstructive Lung Disease accordingly staging of COPD was done. Stage I: Mild COPD; Stage II: Moderate COPD; Stage III: Severe COPD; Stage IV: Very Severe COPD.[7] Patients were selected form medicine out-patient department and controls were chosen those who had no lung disorders and were non-smokers. Patients with history of medical disorders which might interfere with results of the study like diabetes, hypertension, renal and hepatic disease and other pulmonary disorders like asthma, patients who had recent surgery were excluded from the study.

Measurement of laboratory parameters

From each patient 10 ml blood was collected. By centrifugation at 3000 rpm for 10 minutes at room temperature, serum was separated. Analysis was carried out on the samples.

Baker and Frank method for estimation of Serum Vitamin-E: Serum vitamin-E reduces ferric to ferrous ions, which then form a red colored complex with 1 -dipyridyl. Tocopherols and carotenes are first extracted into Xylene and the absorbance is read at 460 nm to measure the carotenes. A correction for the carotenes is made after adding ferric chloride and reading at 520 nm. Results were expressed as mg/dl of vitamin E.[8]

Lowry et al method was used for Serum Vitamin-C level: Vitamin C was determined by titration method which employs the titration with 2, 6-dichlorophenol indophenol in acid solution. On titration with an ascorbic acid solution, this compound is reduced to the colourless leucobase. The ascorbic acid is oxidized to dehydroascorbic acid. End-point was blue to red to colorless. Milligram of vitamin C / 100 ml of plasma are 1.6/ml of titration.[9]

Ernest Beutler et al., method was used for measuring whole blood reduced glutathione: the method is based on the development of a relatively stable yellow color, when 5, 5'- dithio-bis-(2- nitrobenzoic acid) is added to sulfhydryl compounds.1.0 ml of blood extract was mixed with 4.0 ml of 0.3 M Na_2HPO_4 1.0 ml of DTNB reagent [40 mg of 5, 5'- dithiobis (2-nitrobenzoic acid) in 100 ml of aqueous 1% trisodium citrate]. Absorbance was read at 412 nm immediately. Results were expressed as micromoles/liter.[10]

Marklund and Marklund method was used for measuring serum superoxide dismutase (SOD) activity. SOD activity was determined by use a simple and rapid method, based on the ability of the enzyme to inhibit the autoxidation of pyrogallol. The autoxidation of pyrogallol in the presence of EDTA in the pH 8.2 is 50%. The principle of this method is based on the competition between the pyrogallol autoxidation by O_2^- and the dismutation of this radical by SOD.[11]

Serum Nitric oxide (NO) as nitrite was measured by method described by Najwa Cortas and Nabil Wakid. The oxidation products of NO, nitrite (NO_2^-) and subsequently nitrate (NO_3^-), serve as an index of NO production. The method for measuring plasma nitrite and nitrate levels was based on the Griess reaction. Samples were initially deproteinized with Somogyi reagent. Total nitrite (nitrite+nitrate) was measured by spectrophotometry at 545 nm after conversion of nitrate to nitrite by copperized cadmium granule. A standard curve was established from nitrite standards to analyze unknown sample concentrations. Results were expressed as micromoles/liter.[12]

Kei Satoh Method was used for measuring serum malondialdehyde. MDA is being extensively used in assessing the process of lipid peroxidation. Reactive Oxygen Species (ROS) generated causes peroxidation of Polyunsaturated Fatty Acids (PUFA) of the cell membranes. Serum Lipid peroxide was measured by precipitating lipoproteins with trichloroacetic acid and boiled with thiobarbituric acid which reacts with Malondialdehyde to get pink colour.[13]

Statistical Analysis

The results are expressed as mean ± SD of the various parameters measured. SPSS software (2013 version) was used for statistical analysis. For comparing different biochemical parameters between cases and controls, unpaired 't' test was used. The difference was considered significant if the p value was < 0.05.

Results

A total of 84 subjects (42 cases and 42 controls) were included in the final analysis. The demographic data is shown in table 1. There was no statistically significant difference in the demographic data between the cases and controls. Maximum patients were in stage 3 COPD. The anti-oxidant and oxidative stress marker levels between the cases and controls is shown in table 2. There was statistically significant difference in anti-oxidant status and oxidative markers between cases and controls.

Table 1: Demographic data and the pulmonary function tests of cases and controls

Variable	Cases	Controls	P value
Age	48.97 ± 2.32	45.24 ± 2.12	NS
Sex (Male /Female)	34/8	29/13	NS
BMI	29.3±1.8	27.5 ± 1.5	NS
Smokers/ex-smokers	31/13	-	-
COPD stage			
I	03	-	-
II	07	-	-
III	29	-	-
IV	03	-	-

NS= Not significant

Table 2: Anti-oxidant and oxidative stress levels between the cases and controls

Parameter	Cases	Controls	P value
Vitamin C (mg/dl)	0.52 ± 0.06	1.46 ± 0.14	<0.001
Vitamin E (mg/dl)	0.95 ± 0.08	1.59 ± 0.21	<0.001
SOD (U/ml)	4.94 ± 0.74	8.87 ± 1.58	<0.001
GSH (mg/dl)	27.48 ± 1.93	18.27 ± 1.46	<0.001
MDA (μmol/ml)	5.72 ± 1.29	2.54 ± 1.43	<0.001
Serum NO (μmol/L)	112.97 ± 10.56	26.45 ± 5.92	<0.001

SOD = Superoxide dismutase, GSH = Whole blood reduced glutathione, MDA = Malondialdehyde, NO = Nitric oxide.

Discussion

Tobacco smoking is an important cause for development of COPD caused mainly due to reactive oxygen species and lesser extent due to other free radicals.[14] Free radicals act on polyunsaturated fatty acids (PUFA) present on cell membrane and are responsible for lipid peroxidation, as measured by MDA which is used widely as a sensitive marker of lipid peroxidation. In the present study, there was statistically significant (P<0.001) difference in

MDA levels between the cases and controls (table 2). Similar findings have been reported by other studies.[15-18]

Nitric oxide oxidation products are important inflammatory mediators in COPD.[19] Diseases of the pulmonary system such as COPD are characterized by an increased expression of nitric oxide synthase-2.[20] In the present study, a statistically significant difference (p<0.001) was seen in serum nitric oxide levels between the cases (table 2). Similar findings are reported by other studies.[21-22] We did not find other studies which measured serum nitric oxide, but they measured exhaled nitric oxide. Overall there was statically significant difference in oxidative stress levels (MDA and serum nitric oxide levels) between the cases and controls.

The anti-oxidant status as measured by Vitamin C and E, Whole blood reduced glutathione and superoxide dismutase showed statistically significant difference between cases and controls (table 2). Vitamin E is major antioxidant, it protects PUFA from oxidative damage, in addition has anti-inflammatory function, plays a role in maintenance of integrity and fluidity of plasma membranes.[23] Similar findings of significant difference of Vitamin E levels between cases and controls have been reported by other studies.[21,24,25]

A statistically significant difference was also seen with Vitamin C levels between the cases and controls, two reasons postulated are, tobacco smoke stimulates lipid peroxidation in lung that is reduced by vitamin C and vitamin C is required to regenerate vitamin E, similar findings with Vitamin C are reported in other studies.[26-28] One of the important scavengers of super oxide radical is Superoxide dismutase (SOD). The decreased levels of SOD play an important role in the pathogenesis of COPD.[27] In the present study, COPD patients had statistically significant decreased level of Superoxide dismutase when compared with controls (P value < 0.001)). Similar findings with Superoxide dismutase have been reported by other studies.[17,27]

There was a statistically significantly difference in whole blood reduced glutathione levels between the cases and controls. Normally, most of the intracellular glutathione is stored in the reduced form and during oxidative stress, the free sulfhydryl (- SH) groups become oxidized resulting in loss of reduced form. The tobacco smoke (gaseous phase) may also irreversibly react with reduced form (GSH) to form derivatives of GSH, thereby depleting the total available GSH pool.[29] Other studies have also reported similar findings.[30,31]

Limitations of the study

The study was conducted at a single center, we did not include spirometry readings (for pulmonary function tests) and the sample size was small. We did not measure oxidative stress parameters and anti-oxidant status in COPD phenotypes (Chronic bronchitis and Emphysema). There exists evidence for the role of oxidative stress in the pathogenesis of emphysema, mainly in animal studies.[32] Future studies should multi-centric, include large sample size and measure parameters in COPD phenotypes.

Conclusion

The present study revealed that levels of free radicals and oxidative stress markers had shown statistically significant disturbance in the form of increase levels of whole blood reduced glutathione, malondialdehyde, nitric oxide and decreased levels of Vitamin C, Vitamin E, superoxide dismutase among the cases of COPD. The measurement of oxidative stress markers and antioxidant status at regular intervals in COPD patients might be useful in prognosis of the disease. These results may contribute for the development of novel therapeutic interventions. Future research should be directed to know whether anti-oxidant supplementation along with other standard treatments for COPD will significantly alter the prognosis of the disease.

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