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Alteration in Antioxidant Capacity in Patients with Chronic Obstructive Pulmonary Disease

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ABSTRACT

Background: *Chronic obstructive pulmonary disease (COPD) is a major public health problem that needs greater attention. Variability in the susceptibility to develop COPD is related to both genetic and environmental factors. Oxidative stress and inflammation are the major hallmarks of COPD and antioxidant status can be used as a biomarker to assess the risk of chronic diseases.*

Materials and Methods: *We used the FRAP (ferric reducing ability of plasma) assay as a simple and powerful test for determination of the total antioxidant capacity of plasma of patients and normal subjects. The patients were selected by cross-sectional method. The mean average age ±SD of normal subjects and patients was 56 ± 4 and 60±2 years respectively. The spectrophotometeric method was used for this assay.*

Results: *The means of the FRAP assays in the patients were higher (about twice) than those of normal subjects. The differences were significant ($p<0.01$).*

Conclusion: *The high levels of antioxidant capacity in the patient group indicated that the antioxidant defense system had been activated due to the oxidative stress and hypoxic condition. A though, FRAP assay can probably be used for demarcation of severity and risk of developing COPD, clinical follow-up and further investigation are required for the assessment of this hypothesis.* ***(Tanaffos 2007; 6(4): 13-17)***

Keywords*: COPD, Oxidative Stress, Ferric reducing ability of plasma (FRAP), COPD*

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a condition characterized by airway inflammation and progressive and largely irreversible airway obstruction. Cigarette smoking is a major risk factor for COPD. However, only 15 to 20% of cigarette smokers appear to be susceptible to its effects and show a rapid decline in FEV1 and develop the disease. The reason why only some cigarette smokers are susceptible is unclear at present but may relate to differences between their responses to cigarette smoke. Creation of an imbalance between oxidants

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and antioxidants (oxidative stress) is considered to be important in the pathogenesis of COPD. This may be related to "susceptibility" to the oxidative effects of cigarette smoke and hence to the inflammatory response that ensues. This degree of susceptibility is related to genetical differences between persons (1).

The increased oxidative stress in patients with COPD is the result of an increased burden of inhaled oxidants. Oxidants are unstable molecules produced by natural chemical processes missing electrons and tend to bind with other molecules in the body. On the other hand, an antioxidant is defined as a substance that when present in low concentrations compared with that of an oxidizable substrate, significantly delays or reduces oxidation of the substrate by reactive oxygen species (ROS)/reactive nitrogen species (RNS) and other free radicals. The most important ROS of physiological significance are the superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), nitric oxide ($NO^{\bullet}$) and hydrogen peroxide (H_2O_2). The primary ROS formed *in vivo* are $O_2^{\bullet-}$ and H_2O_2. However, the most reactive and harmful of all ROS is the $OH^{\bullet}$. The main cellular sources of ROS in the lung include not only neutrophils, eosinophils and alveolar macrophages, but also alveolar epithelial cells, bronchial epithelial cells and endothelial cells. The generation of ROS in the lungs is also enhanced by smoking (2).

The Ferric reducing ability of plasma (FRAP) assay, is presented as a fast and novel method for assessing "antioxidant power". Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine (TPTZ) complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentrations. Absorbance changes are linear over a wide concentration range with antioxidant mixtures, including plasma, and with solutions containing one antioxidant in the purified form. There is no apparent interaction between antioxidants (3).

The main goal of this study was to evaluate the antioxidant capacity of plasma in COPD patients in comparison with normal subjects by ferric reducing ability of plasma (FRAP) test.

MATERIALS AND METHODS

Population

We studied 42 COPD patients (36 males, 6 females, mean age: 60 ± 2 yrs) in clinically stable conditions (all of them had suffered from an acute exacerbation during, at least, the past 4 months) and 50 healthy (42 males, 8 females, mean age 56 ± 4 yrs) sedentary volunteers of similar age (Table 1). We studied only patients without known neuromuscular disorders, cardiac failure, diabetes mellitus, alcoholism, and those who had exacerbations. All of them signed the informed consent, after being fully aware of the nature, characteristics, and risks of the study. This investigation was approved by the local ethics committee of the hospital (NRITLD).

Table 1. Mean (±SD) of different variables measured in the study.

Variables	Control Subjects	COPD
Age, year	56±4	60±2
FEV1, % reference	90±5	34±3*
FEV1/FVC, %	82±2	43±4*
FRAP (units, μM Fe (II) per liter)	428±37	762±48*

* Significance of differences with respect to control subjects.

Lung Function

Pulmonary function tests (PFT) were performed in all patients. There are some parameters for the assessment of the lung function and grading COPD. Hence, forced expiratory volume produced in the first second (FEV1) and forced vital capacity (FVC) were measured in all participants (4). Spirometric reference values were from a Mediterranean population (5). The patients were categorized as

having mild COPD (FEV1/FVC < 70%, FEV 1 ≥ 80% predicted, moderate COPD (FEV1/IFVC < 70%, 50% ≤ FEV 1 < 80% predicted, severe COPD (FEV1/FVC < 70%, 30% ≤ FEV 1 < 50% predicted and normal (FEV1>75%, FVC% predicted>75%, FEV1/FVC>70%).

Preparing samples

Whole blood was collected by venipuncture in sodium citrate tubes. Blood (7–9 ml) was centrifuged at 400×*g* for 30 minutes. The plasma layer was transferred to a clean 10 ml centrifuge tube and stored at −20 °C for future use as a source for FRAP assay.

FRAP assay

FRAP reagents were prepared as follows: 200 ml acetate buffer, 20 ml TPTZ solution, 20 ml $FeCl_3$ solution and 24 ml distilled water were mixed and kept in water bath at 37˚C. A series of standards were prepared by serial dilution of 1mM ferrous sulphate (0.278g $FeSO_4.7H_2O$ in 1 liter distilled water) to 0.1 mM with distilled water by 0.2 decrease in each standard.

All samples were run at least in triplicate. 1.5 ml of FRAP reagent was added to 30µl of samples and standards and then mixed and incubated at 37°C for 10 minutes. Finally, the absorbance of all samples and standards was read in 593 nm by a double beam spectrophotometer with continuous optical absorbency registry (Shimadzu, Nagoya, Japan).After constructing a linear regression (r^2=0.992) for the standards (absorbance against concentration), we used the regression equation to calculate the FRAP values (units, μM Fe (II) per liter) of the samples (3).

Statistical analysis

Mean and standard deviation (SD), and percentages were used for quantitative and qualitative variables, respectively. Comparisons between the results were carried out using a paired t-test after assessing the normality of the distribution (Kolmogorov- Smirnov test) and the equality of variances (Levine's test) and age effect on FRAP results was normalized by analysis of covariance (ANCOVA) analysis. All analyses were performed using SPSS software version 13. Values of p<0.01 were considered statistically significant.

RESULTS

Severely hemolyzed, lipemic or icteric plasma samples were discarded because of interference with this assay. Sampling was performed 10 h after the last medication. All patients had severe airflow limitation (Table 1). The results of the FRAP assays in the patients compared with normal subjects are shown in Figure 1. The means of the FRAP assays in the patients were higher (about 2 times) than those of normal subjects (Table 1, Figure 2). The differences were significant (p<0.01).

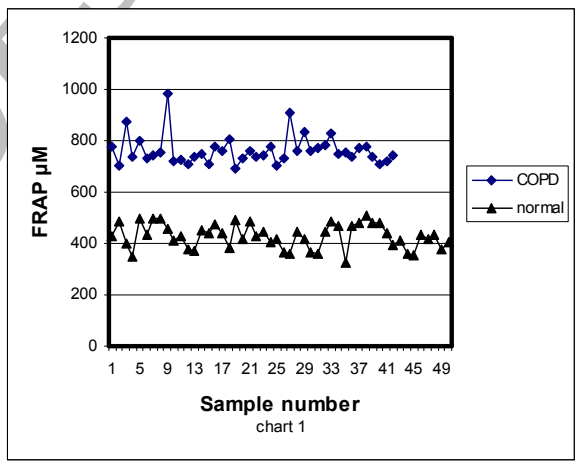

Figure 1. Results of FRAP assay in COPD patients and normal subjects

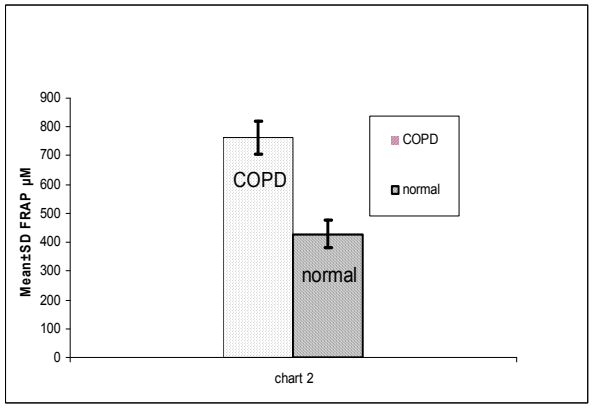

Figure 2. Results of FRAP assay in COPD patients and normal subjects shown as mean ±SD.

DISCUSSION

Several methods (3, 6-12) have been developed to assess the total antioxidant capacity of human serum or plasma. Three assays: the oxygen radical absorbance capacity (ORAC) assay (11), the Randox Trolox-equivalent antioxidant capacity (Randox-TEAC) assay (9), and the ferric reducing ability of plasma (FRAP) assay (3) are the most conventional methods. But for relative determination of total antioxidant capacity, FRAP assay is the best choice. The FRAP assay measures the ferric to ferrous ion reduction in the presence of antioxidants and is very simple and convenient in terms of operation. This assay is fast and inexpensive but does not measure the SH-group-containing antioxidants. The FRAP assay measures the ferric reducing ability of a sample. It is totally different from the ORAC and TEAC assays, because there are no free radicals or oxidants applied in the assay. The antioxidant capacity of an antioxidant against a free radical does not necessarily match its ability to reduce Fe^{3+} to Fe^{2+}. This is why the FRAP value for GSH was almost zero. The use of Fe^{2+} as a final indicator in the FRAP assay may cause problems when an analyzed antioxidant, such as ascorbic acid, not only reduces Fe^{3+} to Fe^{2+}, but can also react with Fe^{2+} to generate additional free radicals(13).

The results of this study demonstrated that the antioxidant capacity of plasma in COPD patients in comparison with normal subjects measured by FRAP assay increased about 2 fold. These results contradict the data of Nadeem et al. (14) who found decreased total antioxidant capacity in the patients with COPD as compared to control subjects.

It should be considered that an efficient antioxidant defense system is important in control of oxidative stress caused by free radicals and other reactive species. Permanent oxidative stress continuously generated in the body of COPD patients (15) results in imbalanced endogenous antioxidant response in these patients.

On the other hand, increased antioxidant capacity is compensative and a logical phenomenon against the predominant oxidative stress in these patients which is consistent with the results obtained in this study.

These results suggest that the total antioxidant capacity of plasma is the part of a tightly regulated homeostatic mechanism. This was expected because an efficient antioxidant defense system is important in the control of oxidative stress caused by free radicals and other reactive species, which are continuously generated in the body of COPD patients. Furthermore, this increase in antioxidant capacity of plasma in COPD patients in comparison with normal subjects might be a primary remedial mechanism against oxidative stress and chronic hypoxic condition in these patients.

In summary, the results of this study indicated that, FRAP assay can probably be used for demarcation of severity and risk of COPD. For the assessment of this hypothesis, clinical follow-up and additional research are needed.

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