*International Journal of Biochemistry Research & Review*
*4(2): 150-162, 2014*

**SCIENCEDOMAIN** *international*
www.sciencedomain.org

# Total Antioxidant Capacity: Correlation with Other Antioxidants and Clinical Utility of Their Levels in Chronic Obstructive Pulmonary Disease

**Vivek Ambade[1*], Alka Sontakke[2] and Dashrath Basannar[3]**

*[1]Department of Biochemistry, AFMC, Pune, 411040, India.*
*[2]Department of Biochemistry, MIMER Medical College, Talegaon, Pune, India,*
*[3]Department of Community Medicine, AFMC, Pune, 411040, India.*

***Authors' contributions***

*Author AS supervised the study, author VA concept, designed, conducted the study, review of literature and wrote the manuscript. Author DB performed all the statistical analysis. All authors read and approved the final manuscript.*

***Original Research Article***

***Received 30th September 2013***
***Accepted 30th December 2013***
***Published 12th January 2014***

## ABSTRACT

**Aims:** Oxidative stress is one of the major pathophysiologic hallmarks in the development of COPD and lung is protected against this by enzymatic and non-enzymatic antioxidants. Total antioxidant activity (TAC), may give more relevant biological information about the individual's overall antioxidant status compared to individual components. This study aims to evaluate correlation between TAC and other antioxidants bilirubin, albumin, urate and ceruloplasmin (CP) and explore the clinical utility of their levels in diagnosis of COPD.
**Study Design:** Comparison study.
**Place and Duration of Study:** Cardio Thoracic Centre, Pune and Department of Biochemistry, AFMC, Pune during Dec 2010 to Aug 2012.
**Methodology:** Study comprised of 86 normals as controls group and 86 confirmed COPD patients as COPD group. CP was estimated by patented kinetic method of Somani and Ambade, while all other analytes were estimated by using commercially available kits.
**Results:** CP was significantly higher in COPD patients (1392.8±281.6 IU/L) as compared to controls (1006.2±236.1 IU/L) while levels of albumin, urate and TAC were significantly

**Corresponding author: Email: vivekambade@rediffmail.com;*

lower in COPD patients. The levels in COPD and controls are albumin: 3.8±0.5 and 4.3± 0.4g/dL; urate: 4.4±1.3 and 5±1.3 mg/dL; TAC: 27.7±6.9 and 36±8.2 mmolTE/L respectively. No appreciable correlation was noticed between any individual antioxidant and TAC.

**Conclusion:** CP and TAC showed statistically significant differences between controls and COPD patients and may have clinical utility in the management of COPD. However, the estimation of TAC is to be done with extreme care.

*Keywords: Chronic obstructive pulmonary disease; antioxidants; oxidative stress; ceruloplasmin; ferroxidase; albumin; urate.*

## 1. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is defined as a condition in which there is chronic obstruction to airflow due to chronic bronchitis and /or emphysema. It is the only chronic disease that has shown progressive upward trend in both mortality and morbidity and is expected to be the third leading cause of death by 2020 [1,2].

Lung with a large surface area is continuously exposed to endogenous or exogenous oxidants [3,4,5] and is protected against oxidative challenge by well-developed enzymatic and non-enzymatic antioxidant systems. Enzymatic antioxidants include superoxide dismutase (SOD), ceruloplasmin, glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rx), catalase etc [6,7], while non-enzymatic antioxidants include glutathione (GSH), ascorbate, urate, α-tocopherol, bilirubin, albumin and lipoic acid. Increased pulmonary exposure to oxidative source and/or reduced antioxidant defenses leads to an imbalance known as oxidative stress [8]. Oxidative stress has important implications on several events of lung physiology and for the pathogenesis of COPD. These include activation of the molecular mechanisms that initiate lung inflammation [9], oxidative inactivation of antiproteases and surfactants, mucus hypersecretion, membrane lipid peroxidation, alveolar epithelial injury, remodeling of extracellular matrix, and apoptosis [10]. In the COPD patients, there also may be a reduction in the endogenous antioxidants as a result of reduction in a Nuclear factor erythroid 2–related factor 2 (NRF2), a transcription factor that regulates several antioxidant genes. Suboptimal NRF2-regulated antioxidant defense in COPD patient lungs accounts for the greater oxidant–antioxidant imbalance leading to COPD [11].

Ceruloplasmin, the major serum inhibitor of lipid peroxidation [12], has been documented as a main extracellular antioxidant in serum [13], inhibiting ferrous ion stimulated lipid peroxidation [14], preventing lung injury, and an abnormality of ceruloplasmin oxidative inhibition could be involved in the pathogenesis of COPD [15]. The antioxidant action has been proposed as a crucial function of ceruloplasmin. Among various substrates, the highest oxidizing activity has been found for $Fe^{+2}$ and thus the alternative name of ferroxidase (EC.1.16.3.1) has been proposed [16,17]. Ceruloplasmin in this study was therefore estimated by its ferroxidase activity, by virtue of which it plays an antioxidant role in COPD.

Urate has been found to be an excellent scavenger of singlet oxygen [18] and protects erythrocyte membrane from peroxidation [19]. Urate was effective in preventing lipid peroxidation at concentrations considerably below those normally found in plasma. Urate acts either by directly scavenging active oxygen species [20,21] or by binding radical-generating transition metals [22]. Antioxidant activity of urate is an important aspect of its in-vivo function [19,23]. Albumin has been considered not only as an antioxidant, but as the

major circulating antioxidant in plasma known to be exposed to continuous oxidative stress [4,24,25]. Ames et al. [26,27] demonstrated that antioxidant effect of bilirubin exceeds that of vitamin E towards lipid peroxidation and levels of serum bilirubin are high enough to account substantially towards total antioxidant capacity of serum [28,29].

The sum of endogenous and exogenous antioxidants represents the total antioxidant activity (TAC). Measuring TAC can provide information on an individual's overall antioxidant status, which may include those antioxidants not yet recognized or not easily measured [30]. Cooperation of all the different antioxidants provides greater protection against attack by reactive oxygen or nitrogen radicals, than any single compound alone. Thus, the overall antioxidant capacity may give more relevant information on an individual's overall antioxidant status compared to that obtained by the measurement of individual components.

To this end, we estimated the levels of TAC, bilirubin, albumin, urate and CP in serum of COPD patients and compared the levels with that in normal subjects, studied correlation of TAC with other antioxidant and their utility in diagnosis of COPD.

## 2. MATERIALS AND METHODS

### 2.1 Subject Selection and Procedure

i) Group I or Controls: Comprises of 86 normal, age and sex matched individuals. Subjects for this group were taken from normal healthy individuals coming to Department of Biochemistry for routine check up and annual medical board and other volunteers.
ii) Group II or COPD patients: Comprises of 86 patients of COPD diagnosed as per GOLD guidelines (GOLD report 2011). For this group, subjects were selected from the patients reporting with symptoms of COPD to respiratory OPD of Cardio-Thoracic Centre, Pune, between Dec 2010 to Aug 2012.

Both the groups were further equally divided into smoker and nonsmoker group (43 smokers and 43 nonsmokers). The clinical assessment at the time of presentation, in terms of signs and symptoms and relevant investigations were recorded in a proforma. Informed consent was taken from the subjects before drawing their blood specimen.

#### 2.2.1 Exclusion criteria for control and COPD patients

Patients with a diagnosis of asthma, coronary artery disease (CAD), diffuse parenchyma lung disease, patients on long term oxygen therapy or unable to perform spirometry were excluded from the Group II. Similarly individuals with any respiratory or lung disease or past history of any lung/respiratory problem/disease were excluded from Group I.

All patients were administered a questionnaire to collect demographic data (name, age, sex, address), duration of symptoms, history of allergic symptoms (history of atopy, nasal allergies), episodic or progressive symptoms, chest examination (wheeze), radiological investigations (CXR, CT Scan), basis of initial diagnosis (ascertained from follow up book) and whether spirometry was performed for initial diagnosis. From the questionnaire an initial clinical impression of the diagnosis was made. Subjects were asked not to use short acting bronchodilators (e.g. beta-agonist salbutamol or anti cholinergic agent ipratropium bromide) within 04 hours of testing, long acting bronchodilators (salmeterol or formoterol) for 12 hours prior to testing and oral therapy with theophyllines or slow release B-agonists for 24 hours prior to the test. All patients underwent spirometry for confirmation of the diagnosis of COPD.

The presence of post bronchodilator FEV1/FVC<0.70 was used to confirm presence of persistent airflow limitation and thus of COPD [31].

Smoking index was used to quantify smoking exposure among the study subjects. Smoking index is defined as number of cigarettes or bidis smoked per day multiplied by the total duration of smoking in years. It is a better index to quantify smoking in Indian context as compared to pack years [32].

## 2.2 Subject Collection and Storage

Fasting venous blood samples, about 5 mL, were collected in a plain sterile gel vacutainer from all the subjects. Serum was separated by centrifuging the blood sample at 2000 rpm for 5 minutes. The supernatant was separated into micro-centrifuge vials/Eppendorf tubes. Estimation of CP, albumin, bilirubin and urate was done immediately without any storage while remaining aliquots were stored frozen at -70$^{\circ}$C in deep freezer until analysed for TAC.

## 2.3 Estimation of Ceruloplasmin

Ceruloplasmin (CP) was estimated by indigenous patented kinetic method of Somani and Ambade (Govt of India Patent No192356), [33] on fully automated analyzer XL 600 from Transasia Mannheim GmbH, Germany, using the reagents as below: Reagent-1: Chromogen (0.5 mmol/L) was made by dissolving 159.65 mg of norfloxacin in 1000 mL of acetate buffer (0.45 mol/L, pH 5.4) containing 0.2% Triton X-100. Reagent-2: Substrate (2.04 mmol/L) was made by sequentially dissolving, 320 mg of DTT and 800 mg of ferrous ammonium sulphate, Fe $(NH4)_2(SO_4)_2 \bullet 6H_2O$, in 1000 mL of deionised water (DIW). The analyser was set at following parameters: Assay Type: Rate A; Wavelength: Primary= 376nm, Secondary=0nm; Assay Points 0, 0, 15, 18 cycles; Sample volume: 10µL; Reagent 1:200µL; Reagent 2:30µL; Factor 2010. This estimation is based on the principle that enzymatic oxidative property, that is, ferroxidase property oxidizes ferrous to ferric ion which then complexes with chromogen. The formation of this complex is measured kinetically at 376nm. The ferroxidase activity in serum was calculated from the factor and displayed directly by the fully automated analyser in IU/L.

## 2.4 Estimation of Albumin, Bilirubin, Urate

Albumin, bilirubin and urate were estimated using kits from Transasia on fully automated analyzer XL 600 from Transasia Mannheim GmbH, Germany. Albumin was estimated by BCG Dye Method, End Point Code No: 120223; Bilirubin by Diazo Method, End Point Code No: 120244 and Urate by Modified – Trinder Method, End Point Code No: 120216.

## 2.5 Estimation of Total Antioxidant Capacity

TAC was estimated using commercially available BioVision Total Antioxidant Capacity (TAC) Assay Kit (Catalog No K274-100); BioVision Research Products, 980 Linda Vista Avenue, CA 94043, USA. It is based on the principle that $Cu^{++}$ ion is converted to $Cu^{+}$ by antioxidants. The reduced $Cu^{+}$ ion is chelated with a colorimetric probe giving a broad absorbance peak around 570nm, proportional to the TAC. As there is no specific substance by the name 'antioxidant', the kit has been provided with Trolox which is the most widely used standard for antioxidant and therefore TAC was measured as TEAC (trolox equivalent antioxidant capacity) in nmol/µL or mmol/L serum.

Kit has been provided with $Cu^{++}$ Reagent, Assay Diluent and Protein Mask. Trolox Standard provided in lyophilized form was dissolved in 20µL of pure Dimethylsulfoxide (DMSO) and made 1mL by DIW to get 1mM solution, which was used to prepare working standard by adding 0, 4, 8, 12, 16, 20µL to individual wells and adjusting the total volume to 100µL with DIW to get 0, 4, 8, 12, 16, 20nmol of working Trolox standard. Kit can be used to estimate TAC or Antioxidant Capacity (AC) due to small molecule. If small molecule AC is desired, serum samples are to be diluted 1:1 with protein mask. Therefore, it is not used in our study. All well volumes were adjusted to 100µL with DIW. Working coloring reagent (prepared by diluting one part $Cu^{++}$ reagent with 49 parts of Assay diluent) of 100µL was added to all standard & sample wells. Plate was incubated at room temperature for 1.5 hours followed by reading the absorbance at 570nm using the plate reader.

After adding all reagents we measured absorbance at different times from 2 minutes to 90 minutes instead of direct 1.5 hours mentioned in the kit. We found that there was no change in the absorbance of the standard (Fig. 1.), but the absorbance in the serum samples was found increasing gradually (Fig. 2). The absorbance of all the serum samples at any time was within the standard curve (between 0.1 to 0.76 as shown in Fig. 2.). From the standard curve which was very identical to that mentioned in the kit insert, regression formula: $y=0.034x+0.050$, $R^2=0.997$ was calculated, from which concentration of TEAC in samples was calculated. The concentration can also be calculated using standard formula of colorimetry. In this study, one of the sample has an absorbance of 0.151, the standard of 4nmol has an absorbance of 0.186, blank was 0.048 & therefore the concentration of TEAC in this sample was 2.985nmol. But this value was for the 0.1µL serum, as in each well 0.1µL serum was added and therefore TEAC of this sample was 29.85nmol/µL or 29.85mmol/L. The absorbance of the same sample after 90 minutes became 0.36 which was near to 8nmol standard with an absorbance of 0.312. Thus same sample TEAC gets calculated as 9.45nmol/0.1µL serum or 94.5mmol/L. It is to be noted that 2 minutes incubation appears more valid, as with time the increase in the absorbance starts deviating. The correlation between absorbance at 2 minutes and 5 minutes ($R^2=0.997$) drops down systematically with time ($R^2=0.84$ at 90 minutes), (Fig. 2.). Finally we decided to use exact 2 minutes incubation time for accurate estimation of serum TAC levels in this study.

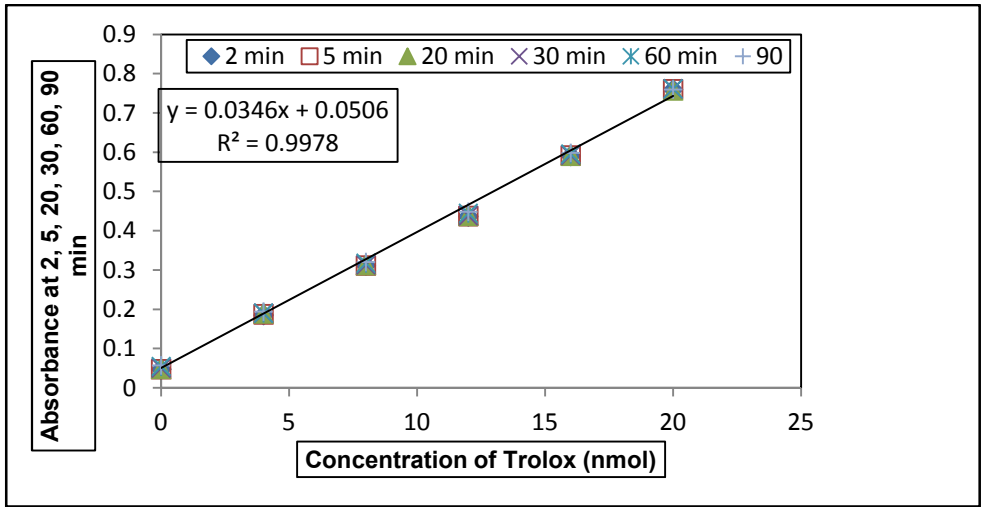


**Fig. 1. Standard curve of Trolox at different times**

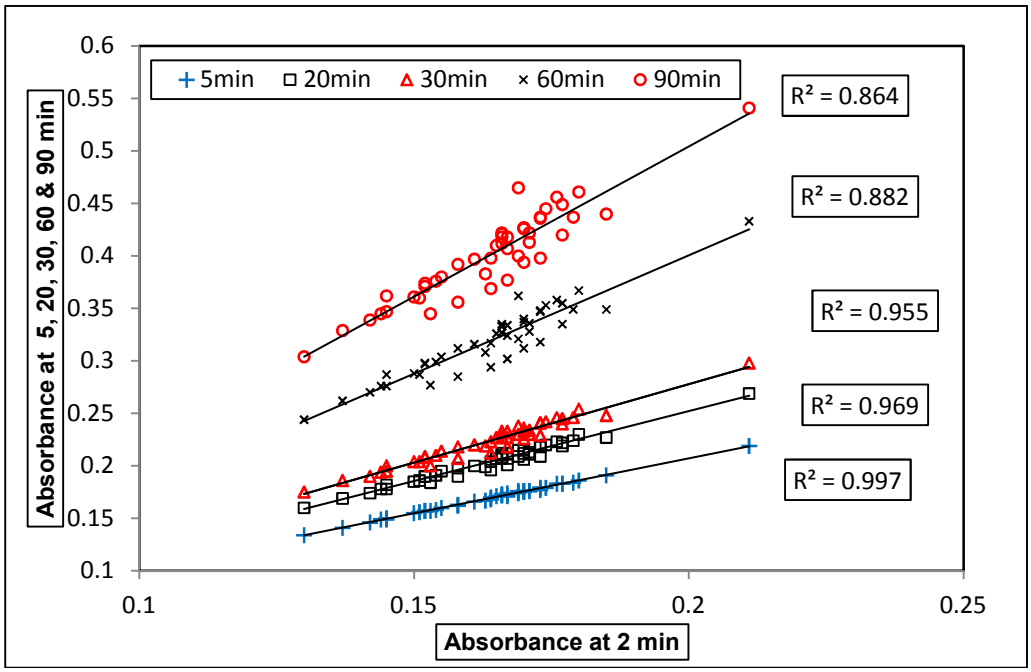


**Fig. 2. Absorbance of serum samples at different times**

## 2.6 Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS version 17). Results have been expressed as mean±SD. Mean differences were tested using unpaired two tailed 't' test. Correlation between the variables was calculated using Pearson's coefficient. ANOVA was applied on the four groups formed, based on disease and smoking status (nonsmoker controls, smoker controls, nonsmoker COPD and smoker COPD). Kolmogorov-Smirnov Test was applied to test normality for each group. ANOVA was applied to compare analyte levels between defined four groups. Post Hoc test (Bonferroni) was applied for multiple comparisons for the analytes where ANOVA was significant. Cut-off point for determining statistical significance levels was *P*=.05.

## 3. RESULTS AND DISCUSSION

In eighty six controls (Group I) male to female ratio was 64:22, Mean±SD of age was 60.9± 11.78 years with range 27-90 years. In eighty six COPD patients (Group II) male to female ratio were 61:25; Mean±SD of age 67.3±8.36 years and range 45-97 years (Table 1). In 43 smoker controls and 43 smokers COPD, male to female ratio was 41:2.

**Table 1. The age and sex distribution of study groups**

| Parameter | Controls (n=86) | | | COPD patients (n=86) | | |
|---|---|---|---|---|---|---|
| | Males | Females | Total | Males | Females | Total |
| Number (%) | 64 (74.4) | 22 (25.6) | 86 (100) | 61 (71) | 25 (29) | 86 (100) |
| Mean (yrs) | 60.8 | 61.2 | 60.89 | 67.3 | 64 | 67.32 |
| SD (yrs) | 13.2 | 5.7 | 11.78 | 8.3 | 7.6 | 8.36 |
| Range (yrs) | 27-90 | 50-77 | 27-90 | 45-97 | 45-76 | 45-97 |

Serum levels of CP were significantly higher in COPD patients as compared to controls. Mean±SD of CP in COPD versus controls respectively is 1392.8±81.6 IU/L versus 1006.2±236.1 IU/L; (Table 2). This is in agreement with the study by Verrills et al [34], who found that a panel of four biomarkers (α(2)-macroglobulin, haptoglobin, ceruloplasmin, and hemopexin) was able to discriminate with statistical significance between the clinical groups of patients with asthma, patients with COPD, and control subjects and reported increased ceruloplasmin in COPD patients as compared to controls. Serum levels of albumin, urate and TAC were significantly lower in COPD patients as compared to controls. Levels of albumin, urate and TAC in COPD versus controls respectively are albumin: 3.8±0.5 g/dL versus 4.3±0.4g/dL; urate: 4.4±1.3mg/dL versus 5±1.3mg/dL; TAC 27.7±6.9mmolTE/L versus 36±8.2mmolTE/L (Table 2). The fall in albumin in COPD in our study is similar to that reported by Moison et al. [35]. Kolmogorov-Smirnov test for testing the normality of each analyte in each of the four groups namely nonsmoker controls, smoker controls, smoker COPD and nonsmoker COPD was applied and was found not significant for all the analyte (P>0.05) (Table 3). ANOVA was accordingly applied to all the analytes. Except total bilirubin (P=0.9), all the other four analytes were found to have significant differences between all four groups (Table 4). Post Hoc Test– Bonferroni was applied to these analytes for multiple comparisons between four groups. No significant difference was found in albumin and urate between smoker and nonsmoker group either within controls or COPD (Table 5). This is in agreement with that of Lykkesfeldt et al. [36]. CP levels were found significantly different between all the groups except nonsmoker and smoker control and nonsmoker and smoker COPD, while TAC was found significantly different between all groups, except between smoker control and nonsmoker COPD (Table 5). The significantly

**Table 2. Comparison of levels of different analytes between controls and COPD**

| Analyte | Controls (n=86) | | COPD patients (n=86) | | significance |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | P value |
| Total Antioxidant capacity (mmolTE/L) | 36.0 | 8.2 | 27.7 | 6.9 | <0.001 |
| Albumin (g/dL) | 4.3 | 0.4 | 3.8 | 0.5 | <0.001 |
| Total Bilirubin (mg/dL) | 0.8 | 0.4 | 0.8 | 0.4 | 0.899 |
| Uric acid (mg/dL) | 5.0 | 1.3 | 4.4 | 1.3 | 0.031 |
| Ceruloplasmin (IU/L) | 1006.2 | 236.1 | 1392.8 | 281.6 | <0.001 |

**Table 3. Descriptive statistics showing One-Sample Kolmogorov-Smirnov Test**

| Biomarker | Nonsmoker controls(n=43) | | | | | |
|---|---|---|---|---|---|---|
| | Mean | SD | SE | 95% CI for mean | Kolmogorov-Smirnov Z | Kolmogorov-Smirnov Sig (2 tailed) |
| Total Antioxidant capacity (mmolTE/L) | 38.1 | 8.1 | 1.24 | 35.6-40.6 | 0.65 | 0.79 |
| Albumin (g/dL) | 4.3 | 0.2 | 0.04 | 4.2- 4.4 | 0.75 | 0.63 |
| Total Bilirubin (mg/dL) | 0.8 | 0.4 | 0.06 | 0.7-0.9 | 0.89 | 0.41 |
| Uric acid (mg/dL) | 4.9 | 1.4 | 0.21 | 4.5-5.3 | 0.68 | 0.74 |

***Table 3 Continue.....***

| | | | | | | |
|---|---|---|---|---|---|---|
| Ceruloplasmin (IU/L) | 979.2 | 206.6 | 31.51 | 915.6-1042.8 | 0.54 | 0.93 |
| **Smoker controls (n=43)** | | | | | | |
| Total Antioxidant capacity (mmolTE/L) | 33.9 | 7.8 | 1.20 | 31.5-36.5 | 0.62 | 0.84 |
| Albumin (g/dL) | 4.3 | 0.5 | 0.08 | 4.2-4.5 | 1.23 | 0.1 |
| Total Bilirubin (mg/dL) | 0.8 | 0.4 | 0.06 | 0.7-0.9 | 0.82 | 0.51 |
| Uric acid (mg/dL) | 5.1 | 1.3 | 0.20 | 4.7-5.5 | 0.4 | 1.0 |
| Ceruloplasmin (IU/L) | 1033.2 | 262.0 | 39.96 | 952.5-1113.8 | 0.60 | 0.86 |
| **Nonsmoker COPD (n=43)** | | | | | | |
| Total Antioxidant capacity (mmolTE/L) | 31.8 | 5.1 | 0.78 | 30.3-33.4 | 1.1 | 0.18 |
| Albumin (g/dL) | 3.8 | 0.5 | 0.08 | 3.6-3.9 | 0.91 | 0.38 |
| Total Bilirubin (mg/dL) | 0.8 | 0.5 | 0.08 | 0.7-1.0 | 0.95 | 0.32 |
| Uric acid (mg/dL) | 4.6 | 1.2 | 0.19 | 4.2-5.0 | 0.66 | 0.77 |
| Ceruloplasmin (IU/L) | 1350.3 | 294.2 | 44.87 | 1259.7-1440.8 | 1350.3 | 294.2 |
| **Smoker COPD (n=43)** | | | | | | |
| Total Antioxidant capacity (mmolTE/L) | 23.6 | 5.9 | 0.90 | 21.8-25.4 | 0.49 | 0.97 |
| Albumin(g/dL) | 3.8 | 0.5 | 0.08 | 3.6-3.9 | 1.0 | 0.27 |
| Total Bilirubin (mg/dL) | 0.8 | 0.3 | 0.05 | 0.7-0.9 | 0.87 | 0.44 |
| Uric acid(mg/dL) | 4.3 | 1.4 | 0.21 | 3.7-4.7 | 0.5 | 0.96 |
| Ceruloplasmin (IU/L) | 1435.3 | 264.9 | 40.39 | 1353.8-1516.8 | 0.73 | 0.67 |

**Table 4. ANOVA test for analytes**

| Dependent Variable | Group | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Total Antioxidant Capacity | Between Groups | 4765.8 | 3 | 1588.59 | 33.68 | 0.00 |
| | Within Groups | 7925.2 | 168 | 47.17 | | |
| Albumin | Between Groups | 12.8 | 3 | 4.26 | 18.52 | 0.00 |
| | Within Groups | 38.7 | 168 | 0.23 | | |
| Total Bilirubin | Between Groups | 0.1 | 3 | 0.03 | 0.20 | 0.90 |
| | Within Groups | 27.9 | 168 | 0.17 | | |
| Urate | Between Groups | 15.8 | 3 | 5.26 | 3.02 | 0.03 |
| | Within Groups | 292.7 | 168 | 1.74 | | |
| Ceruloplasmin | Between Groups | 6644983.6 | 3 | 2214994.53 | 33.05 | 0.00 |
| | Within Groups | 11259730.8 | 168 | 67022.21 | | |

**Table 5. Post Hoc Test – Bonferroni: Multiple comparisons between all four groups (smoker controls, nonsmoker controls, smoker COPD and nonsmoker COPD)**

| Dependent Variable | Group (x) | Group (y) | Mean Difference between groups (x-y) |
|---|---|---|---|
| Total Antioxidant Capacity | Nonsmoker Controls | Smoker Controls | 4.17* |
| | | Nonsmoker COPD | 6.22* |
| | | Smoker COPD | 14.46* |
| | Smoker Controls | Nonsmoker COPD | 2.04 |
| | | Smoker COPD | 10.28* |
| | Nonsmoker COPD | Smoker COPD | 8.23* |
| Albumin | Nonsmoker Controls | Smoker Controls | -0.051 |
| | | Nonsmoker COPD | 0.51* |
| | | Smoker COPD | 0.52* |
| | Smoker Controls | Nonsmoker COPD | 0.565* |
| | | Smoker COPD | 0.576* |
| | Nonsmoker COPD | Smoker COPD | 0.009 |
| Urate | Nonsmoker Controls | Smoker Controls | -0.228 |
| | | Nonsmoker COPD | 0.305 |
| | | Smoker COPD | 0.576 |
| | Smoker Controls | Nonsmoker COPD | 0.531 |
| | | Smoker COPD | 0.802* |
| | Nonsmoker COPD | Smoker COPD | 0.271 |
| Ceruloplasmin | Nonsmoker Controls | Smoker Controls | -53.93 |
| | | Nonsmoker COPD | -371.06* |
| | | Smoker COPD | -456.08* |
| | Smoker Controls | Nonsmoker COPD | -317.12* |
| | | Smoker COPD | -402.15* |
| | Nonsmoker COPD | Smoker COPD | -85.025 |

** The mean difference is significant at the 0.05 level*

lower serum levels of TAC in COPD patients as compared to controls is in agreement with that of Sahin et al. [37] who reported lower antioxidant capacity in COPD. The lower levels of TAC may be due to decreased synthesis of antioxidants or increased consumption of antioxidants by oxidants or may be by oxidative modifying the antioxidant property of the antioxidant by oxidative stress [38]. In nonsmokers, exposure to smoke from biomass fuel has been suggested as cause for the COPD [39].

Most of the published studies reported the TAC in trolox equivalent (TE) per liter and mentioned levels as TEAC (trolox equivalent antioxidant capacity). The levels of TAC in healthy controls reported in various studies are 0.77±0.09mM/L [40], 1.69±0.2mmol/L [41], 675±61µmolTE/L [42], 12048±2678µmolTE/L [43], 1.0±0.6µmolTE/L [44], 1.31mmol/L in healthy nonsmokers, 0.87mmol/L in healthy smokers [45]. Thus the values from the published studies have been seen ranging from 1µmolTE/L to 12048µmolTE/L. This variation might be due to different methodologies used to estimate TAC [46]. In our study the values were 36±8.2mmolTE/L. We looked into the methodology of the kit used (TAC Assay Kit, BioVision, USA) and first standardized the incubation time to exact 2 minutes so as to minimize the errors and variation due to differences in the incubation time.

As it is reported that, the contributions to TAC are provided by albumin and urate [47-49], so correlation studies between TAC and albumin, urate and bilirubin were conducted, and found

that none of the parameters have any considerable correlation with TAC in controls (r=0.11, 0.01 and 0.008 respectively) or in COPD (r=0.017, 0.112 and 0.037 respectively), though albumin and urate were also significantly lower in COPD patients as compared to controls.

Thus the changes in the levels of TAC could not be correlated with proportionate changes in albumin, urate and bilirubin. This correlation was also studied in smoker and nonsmoker within controls and COPD, and no noticeable correlation was found. Also, no correlation was found between CP and TAC either in controls or COPD. This suggests that TAC is not limited to albumin, urate, bilirubin, CP but due to any other enzymatic or no enzymatic antioxidants. There are studies where TAC has been reported to correlate positively with vitamin E levels (P=0.05), GSH (P=0.02) but not with vitamin C [40]. The depletion of plasma GSH has been suggested as a key point in depletion of other antioxidants [50].

Clinical trials directed toward enhancing lung antioxidants using direct antioxidant molecules, such as vitamin E, showed almost no effect on $FEV_1$ indicating a single antioxidant molecule not efficacious in affording protection against the oxidative stress in COPD and accordingly combination of antioxidants were suggested effective in the treatment of COPD [10]. Strategy based on targeting NRF2, which up-regulates a wide range of antioxidants genes, was suggested as a more efficient COPD therapy [11]. These experiments also indirectly favor TAC measurement over an individual antioxidant in the COPD diagnosis.

## 4. CONCLUSION

CP was significantly increased while TAC gets significantly decreased in COPD. CP and TAC showed statistically significant differences between controls and COPD patients and may have clinical utility in the management of COPD. However, the estimation of TAC is to be done with extreme care. Study with enough patients in each COPD GOLD group may further link the clinical utility of these antioxidant measurements.

## CONSENT

Declare that written informed consent was obtained from the patient for sample.

## ETHICAL APPROVAL

All authors hereby declare that the study was approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Obtained necessary Institutional ethical approval.

## ACKNOWLEDGEMENTS

I sincerely acknowledge the efforts taken by Dr Rahul Tyagi, of Cardio-thoracic centre for correct diagnosis of COPD. I thank Mr. Rubesh KR, Dept of Biochemistry for carrying out the analyses by fully automated clinical chemistry analyser.

## COMPETING INTERESTS

Authors declare that no competing interests exist.

## REFERENCES


1. Jindal SK, Aggarwal AN, Gupta D. A review of population studies from India to estimate national burden of COPD and its association with smoking. Ind J Chest Dis Allied Sci. 2001;43:139-47.
2. Behera D. Respiratory Medicine in India in the 21st Century. Ind J Chest Dis Allied Sci. 2008;50:179-181.
3. Fernando H, Khatri S, David MG. Antioxidant treatment for lung diseases. Expert Opinion on Therapeutic Patents 2005; 15(12): 1711-25.
4. Halliwell B. Antioxidants in human health & disease. Annu Rev Nutr. 1996;14:33-50.
5. MacNee W. Treatment of stable COPD: Antioxidants. Eur Respir. 2005;14(94):12-22.
6. Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M et al. Functional food science and defence against reactive oxidative species. British J of Nutrition. 1998;80:77-112.
7. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease an overview. Methods Enzymol. 1998;186:1-85.
8. Jindal SK, Aggarwal AN, Gupta D. A review of population studies from India to estimate national burden of COPD and its association with smoking. Ind J Chest Dis Allied Sci. 2001;43:139-47.
9. Rahman I, MacNee W. Role of transcription factors in inflammatory lung diseases. Thorax. 1998;53:601-12.
10. Rahman I. The role of oxidative stress in the pathogenesis of COPD implications for therapy. Treat Respir Med. 2005;4(3):175-200.
11. Malhotra D, Thimmulappa R, Navas-Acien A, Sandford A, Elliott M, Singh A et al. Decline in nrf2-regulated antioxidants in chronic obstructive pulmonary disease lungs due to loss of its positive regulator, DJ-1. Am J Respir Crit Care Med. 2008;178(6):592–604.
12. Galdston M, Levytska V, Schwartz MS, Magnusson B. Ceruloplasmin increased serum concentration and impaired antioxidant activity in cigarette smokers, and ability to prevent suppression of elastase inhibitory capacity of α1-proteinase inhibitor. The American Rev of Resp Dis. 1984;129(2):258-63.
13. Dormandy TL. Free radical oxidation and antioxidants. Lancet. 1978;1:647-50.
14. Osaki S, Johnson D, Frieden E. The possible significance of the ferrous oxidase activity of ceruloplasmin in normal human serum J. Biol Chem. 1966;241:2746-51.
15. Taylor JC, Madison R, Wong E, Mittman C. Do Ceruloplasmin and Elastase inhibition abnormalities influence the pathogenesis of chronic obstructive pulmonary disease. Chest. 1984;8(6):45.
16. Young SN, Curzon GA. Method for obtaining linear reciprocal plots with ceruloplasmin and its application in a study of the kinetic parameters of ceruloplasmin substrates. Biochem J. 1972;129:273-83.
17. Curzon G. Some properties of coupled iron ceruloplasmin oxidation systems. Biochem J. 1961;79:656-63.
18. Simon MI, Vanvunakis H. The dye-sensitized photooxidation of purine and pyrimidine derivatives. Arch Biochem Biophys. 1964;105:197–206.
19. Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant and radical-caused aging and cancer a hypothesis. Proc Nat Acad Sci. 1981;78(11):6858-62.
20. Arthur JH, Brenda D, David E, John PP. Urate-null rosy mutants of Drosophila melanogaster are hypersensitive to oxygen stress. Proc Nat Aca Sci. 1992;89:4343-47.

21. Kaur H, Halliwell B. Action of biologically relevant oxidizing species upon uric acid. Identification of uric acid oxidation products. Chem-Biol Interact. 1990;73:235-247.
22. Davies KJ, Sevanian A, Muakkassah-Kelly SF, Hochstein P. Uric acid-iron ion complexes a new aspect of the antioxidant functions of uric acid. Biochem J. 1986;235(3):747-54.
23. Cutler RG. Antioxidants, Aging, and Longevity. In Pryor WA (Ed), Free Radicals in Biology, Vol. VI, Academic Press, Inc. (ISBN 0-12-566506-7). 1984;371-428.
24. Soriani M, Pietraforte D, Minett IM. Antioxidant potential of anaerobic human plasma role of serum albumin and thiols as scavengers of carbon radicals. Arch Biochem Biophys. 1994;312:180–8.
25. Cha MK, Kim IH. Glutathione-linked thiol peroxidase activity of human serum albumin a possible antioxidant role of serum albumin in blood plasma. Biochem Biophys Res Commun. 1996;222:619–25.
26. Stocker R, Glazer AN, Ames BN. Antioxidant activity of albumin-bound bilirubin. Proc Natl Acad Sci. 1987;84:5918–5922.
27. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin an antioxidant of possible physiological importance Science.1987;235:1043–46.
28. Belanger S, Lavoie JC, Chessex P. Influence of bilirubin on the antioxidant capacity of plasma in newborn infants. Biol Neonate. 1997;71:233–238.
29. Gopinathan V, Miller NJ, Milner AD, Rice-Evans CA. Bilirubin and ascorbate antioxidant activity in neonatal plasma. FEBS Let. 1994;349:197–200.
30. Lamonta J, Campbell J, FitzGerald P. Measurement of Individual vs Total Antioxidants Clinical Chemistry. 1997;43(5):852-54.
31. Jindal SK, Malik SK. Smoking index a measure to quantify cumulative smoking exposure Lung. India. 1988;4:195-6.
32. Global initiative for Chronic Obstructive Lung Disease (GOLD). Update. www.goldcopd.org; 2011.
33. Somani BL, Ambade Vivek. A kinetic method amenable to automation for ceruloplasmin estimation with inexpensive and stable reagents. Clinical Biochemistry. 2007;40:571–574.
34. Verrills NM, Irwin JA, He XY, Wood LG, Powell H, Simpson JL, et al. Identification of novel diagnostic biomarkers for asthma and chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2011;183(12):1633-43.
35. Moison RMW, Haasnoot AA, Zoeren-Grobben DV, Berger HM. Plasma Proteins in Acute and Chronic Lung Disease of the Newborn: A descriptive study. Free Radical Biology and Medicine. 1998;(25)3:321-28.
36. Lykkesfeldt J, Viscovich M, Henrik EP. Plasma malondialdehyde is induced by smoking a study with balanced antioxidant profiles British J of Nutrition 2004;92:203-6.
37. Sahin U, Unlu M, Ozguner F, Sutcu R, Akkaya A, Delibas N. Lipid peroxidation and gluathione peroxidase activity in chronic obstructive pulmonary disease exacerbation prognostic value of malondialdehyde. J Basic Clin Physiol Pharmacol. 2001;12:59-68.
38. Olivieri S, Conti A, Lannaccone S, Cannistraci CV, Campanella A, Barbariga M et al. Ceruloplasmin oxidation, a feature of Parkinson's disease CSF, inhibits ferroxidase activity and promotes cellular iron retention. J Neurosci. 2011;31(50):18568-77.
39. Salvi S, Barnes PJ. Chronic obstructive pulmonary disease in non-smokers. The Lancet. 2009;374(9691):733 - 43.
40. Selvi R, Angayarkanni N, Biswas J, Ramakrishnan S. Total antioxidant capacity in Eales' disease, uveitis and cataract. Ind J Med Res. 2011;134:83-90.
41. Dasgupta A, Malhotra D, Levy H, Marcadis D, Blackwell W, Johnston D. Decreased total antioxidant capacity but normal lipid hydroperoxide concentrations in sera of critically ill patients. Life Sci. 1997;60(4-5):335-40.

42. Alessandra B, Claudio C, Mauro R, Pier-Giacomo B, Domenico O. Oxidative stress and total antioxidant capacity in human plasma. Redox Report. 2009;14(3):125-131.
43. Gianna F, Tiziana B, Letizia S, Nicola M, Cinzia C, Antonio B, et al. Lipid Peroxidation and Paraoxonase-1 Activity in Celiac Disease Article ID 587479. J of Lipids. 2012;1–7.
44. Alpay C, Dost Z, Ali A, Sahabettin S, Ozcan E. Oxidative status and paraoxonase activity in children with asthma. Clin Invest Med. 2009;32(5):327-334.
45. Rahman I, Swarska E, Henry M, Stolk J, MacNee W. Is there any relationship between plasma antioxidant capacity and lung function in smokers and in patients with chronic pulmonary disease? Thorax. 2000;55:189-93.
46. Eugene HJM, Ruskovska T. Comparative analysis of serum (anti)oxidative status parameters in healthy persons. Int J Mol Sci. 2013;14:6106-15.
47. Wayner DD, Burton GW, Ingold KU, Barclay LR, Locke Si. The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma. Biochim Biophys Acta. 1987;924:408-19.
48. Miller N, Rice-Evans C, Davies M, Gopinathan V. Mimer A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci. 1993;84:407-12.
49. Jackson P, Loughrey CM, Lightbody JH, McNamee PT, Young IS. Effect of hemodialysis on total antioxidant capacity and serum antioxidants in patients with chronic renal failure. Clin Chem. 1995;41:1135-8.
50. Bose KC, Vyas P, Singh M. Plasma non-enzymatic antioxidants-vitamin C, E, β-carotenes, reduced glutathione levels and total antioxidant activity in oral sub mucous fibrosis. European Rev Medical and Pharmacological Sciences. 2012;16:530-32.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sciencedomain.org/review-history.php?iid=390&id=3&aid=3289*