

# Trace elements and oxidative stress in chronic obstructive pulmonary disease

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## ABSTRACT

**Objectives:** Many trace elements have activator or inhibitor roles in the antioxidative defense systems in diseases such as chronic obstructive pulmonary diseases (COPD). In this study, we aimed to show the levels of trace elements with action in oxidative stress, and to show the levels of malondialdehyde (MDA) as a marker of oxidative stress, and the activity of paraoxonase (PON1) as an antioxidant in COPD and smokers.

**Methods:** We included 25 patients with COPD, and 20 healthy non-smokers in the study. We selected them from the hospitalized patients at the Hospital of Dicle University, Turkey, between April 2003 and January 2004. The clinical condition of the patients was stable.

**Results:** The serum copper (Cu) and MDA concentrations in COPD patients were higher than the

control group. There were no differences in zinc (Zn) concentration and Cu/Zn ratio between COPD patients and the control group. We found lower serum PON1 activities in COPD patients compared with the control group. There was no statistically significant difference in Zn concentration and Cu/Zn ratio between smokers and non-smokers in COPD. There were statistically significant differences in Cu, MDA concentrations and serum PON1 activities between smokers and non-smokers in COPD.

**Conclusions:** We could suggest that trace elements such as Cu, oxidants and antioxidants such as MDA and PON1 have roles in oxidative stress, and in COPD.

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Oxidative stress plays an important role in the pathogenesis of many diseases such as chronic obstructive pulmonary diseases (COPD).<sup>1</sup> One of the main causes of oxidative stress in COPD is cigarette smoking because tobacco contains many compounds which are oxidants and prooxidants.<sup>1-3</sup> An oxidant and antioxidant imbalance in favor of oxidants may cause oxidative damage on air space epithelial cells.<sup>4</sup> Lipid peroxidation occurs at the cell membrane by the effect of free radicals generated by smoking.<sup>2</sup> Malondialdehyde (MDA) is the last product of lipid peroxidation and is a marker of oxidative stress.<sup>5,6</sup> Clara cell is one of the oxidant resistant airway cell in all species, and secretions of Clara cells play a role in protection from oxidative

stress.<sup>7</sup> Mango et al<sup>7</sup> showed a protective role for Clara cells and their secretions in mice, and an effective role in susceptibility to oxidant stress. Mucous cells replace Clara cells in smokers and there is a reduction in Clara cells in COPD and smokers.<sup>8</sup> Paraoxonase (PON1) EC.3.1.8.1, an antioxidant enzyme hydrolyze paraoxon, is a potent inhibitor of the cholinesterases and localizes in Clara cells, endothelial cells and type 1 cells of alveolar epithelium. In the lung, PON1 may have a role in protecting from oxidative stress,<sup>9</sup> however, smoking which causes vital damage at the airspace epithelium, may also cause reduction in levels of PON1.<sup>9,10</sup> The results of studies suggest that several environmental factors can influence serum PON1 concentration

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and activity, and we can associate smoking with reduced serum PON1.<sup>10</sup> In the light of these studies and findings, we decided to investigate the antioxidant capacity of COPD patients, and the relationship between smoking by measuring activities of PON1 in serum. Trace elements have important roles in activation or inhibition of enzymatic reactions. They compete with other elements and metalloproteins and affect the permeability of cell membranes. In the light of this knowledge, trace elements affect the pathogenesis of several diseases such as COPD.<sup>11,12</sup> Trace element concentrations change in smokers, and tobacco smoking can affect antioxidant enzyme activities, thereby indirectly affecting trace element metabolism.<sup>6,13</sup> In this study, we have 2 main aims: a) to investigate the levels of trace elements with action in oxidative stress, and b) to show the levels of MDA as a marker of oxidative stress and activities of PON1, which may have a role as an antioxidant in COPD.

**Methods.** The study population consisted of 25 patients (male/female: 9/16) with COPD diagnosed according to GOLD 2003 guidelines.<sup>3</sup> They were selected from the hospitalized patients at the Hospital of Dicle University, Turkey, between April 2003 and January 2004. The clinical condition of the patients was stable. The control population consisted of 20 healthy, non-smoker volunteers with no history of lung disease. The patients having any criteria of acute exacerbation were excluded from the study group. Having any other pulmonary disease and other systemic diseases were the criteria of exclusion from the control group. The patients having any criteria of acute exacerbation were excluded from the study group. Having any other pulmonary disease and other systemic diseases were the criteria of exclusion from the control group. Blood samples (approximately 10 ml) were collected by venipuncture in plain tubes, that they were obtained by centrifugation at 4000 x g for 10 minutes at room temperature. The samples were stored at -20°C for one week. Serum concentrations of copper (Cu) and zinc (Zn) were measured using a flame atomic absorption spectrophotometer (AA-6401 F SHIMADZU). The levels of serum lipid peroxidation products such as thiobarbituric acid (TBA-MDA) were measured spectrophotometrically by the described modified Buege method.<sup>14</sup> The concentrations of lipid peroxidation products were calculated as MDA concentration using the extinction coefficient for the MDA-thiobarbituric acid complex of  $1.56 \times 10^5$  L/mol cm at 535 for calculation. The levels of serum PON1 were

measured spectrophotometrically by modified Eckerson method. Initial rates of hydrolysis of paraoxon (0,0-diethyl-0-p-nitrophenylphosphate; Sigma Chemical Co, London) were determined by measuring liberated-p-nitrophenol at 405 nm at 37°C.<sup>15</sup> One unit of paraoxonase activity was described as the amount of PON producing 1 micromol of p-nitrophenol per minute litre of serum (mmol/min.L). Spectrophotometric measurements were carried out with Shimadzu UV-1208 spectrophotometer.

**Statistical analysis.** Results were expressed as mean  $\pm$  SD. The differences between the 2 groups were examined using Mann-Whitney U test, and correlations between variables were assessed using Pearson's correlation coefficients. A *p*-value of  $<0.05$  was considered statistically significant.

**Results.** The demographic data of the study population and results of respiratory function test are shown on **Table 1**. The levels of Cu were significantly higher in the patients with COPD compared with the samples in the control group ( $p < 0.01$ ). There were no statistically significant differences between the patients with COPD and control group according to the levels of Zn and Cu/Zn. The serum concentrations of MDA were significantly higher in patients with COPD compared with the control group. The serum activities of PON1 were significantly lower in the patients with COPD compared with the control group. The results are presented in **Table 2**. There were no statistically significant differences between the levels of Zn and Cu/Zn in smokers and non-smokers in COPD. There were statistically significant differences between the levels of Cu, MDA and PON1 activities in smokers and non-smokers in COPD. The serum concentration of MDA, Cu, Zn and Cu/Zn ratio and serum activities of PON1 in smokers and non-smokers with COPD are presented in **Table 3**.

**Discussion.** We know that Cu and Zn are cofactors in many biochemical reactions. Copper is a cofactor of many enzymes and behaves as a catalyst in the production of free radicals and lipid peroxidation.<sup>16,17</sup> Zinc is another trace element which we can find in active sites of superoxide dismutase, an enzyme of antioxidative system. This enzyme protects cells from the events causing formation of free radicals such as ionizing radiation, antibiotics, and anticancer drugs. In light of this, there are previous studies on trace elements in COPD. Molteni et al<sup>18</sup> found serum copper levels within the normal levels in COPD patients. In another study, Tadzhev<sup>19</sup> found that serum Cu and Zn levels returned to normal with treatment by Zn sulphate. Karadag et al<sup>6</sup> found high Cu levels and lower Zn levels in COPD patients compared with a control

Table 1 - The demographic data of the study population.

Demographic data	COPD (n=25)	Control (n=20)
Age*	61.22±10.85	52.84±9.90
Male/female	9/16	9/11
Smoker	12	-
Non-smoker	13	20
Cigarette pack years*	55.17±15.61	-
FEV <sub>1</sub> (%)*	34.44±13.11	81.30±12.05
FEV <sub>1</sub> /FVC (%)*	61.28±7.75	85.65±7.90

\*Result were expressed as mean ±SD.  
FEV<sub>1</sub> - forced expiratory volume, FVC - forced vital capacity,  
COPD - chronic obstructive pulmonary diseases.

Table 2 - The serum concentration of malondialdehyde (MDA), copper (Cu), zinc (Zn), Cu/Zn ratio and serum paraoxonase (PON1) activities of patients with chronic obstructive pulmonary diseases and the control group.

Patients	MDA (nmol/ml)	PON1 (U/L)	Cu (ppm)	Zn (ppm)	Cu/Zn
Control (n=20)	1.33±0.72	107.84±36.33	0.73±0.22	0.77±0.21	1.39±0.71
COPD* (n=25)	2.21±0.79	49.75±27.08	1.03±0.51	0.79±0.25	1.38±0.74
P-value	<0.005	<0.005	<0.01	>0.05	>0.05

\*Result were expressed as mean ±SD.

Table 3 - The serum concentration of malondialdehyde (MDA), copper (Cu), zinc (Zn), Cu/Zn ratio and serum paraoxonase (PON1) activities of smokers and non-smokers with chronic obstructive pulmonary diseases.

Sample	MDA (nmol/ml)	PON1 (U/L)	Cu (ppm)	Zn (ppm)	Cu/Zn
Smoker (n=12)	2.68±0.89	25.63±9.31	0.913±0.40	0.817±0.45	1.29±0.73
Non-smokers* (n=25)	1.78±0.31	72.02±16.42	1.075±0.60	0.70±0.16	1.46±0.79
P-value	<0.001	<0.001	<0.05	>0.05	>0.05

\*Result were expressed as mean ±SD.

group. In a rat model, Ward et al<sup>20</sup> investigated serum Cu levels as an index of lung injury. In our study, we also found high serum Cu levels in COPD patients compared with normal subjects, but contrary to Karadag's study,<sup>6</sup> there were no significant differences in patients with COPD and control group according to the levels of Zn and Cu/Zn ratio. Although there were no significant differences in the levels of both Zn and Cu/Zn ratio between smokers and non-smokers in COPD patients, the levels of Cu were lower in smokers compared with non-smokers. However, we still believe that the number of patients should have been more with respect to smoking status, and nutritional intake must be taken into account, which we did not do. Rahman et al<sup>13</sup> demonstrated that the products of lipid peroxidation increased, and antioxidant capacity decreased in COPD patients and smokers. In the same study, they also found higher levels of lipid peroxidation products in healthy smokers and lower levels in healthy non-smokers.<sup>13</sup> Colak et al<sup>21</sup> also showed that the levels of MDA were higher in COPD patients than healthy controls. We also showed higher levels of MDA in our study, a last product of lipid peroxidation, in COPD and in smoker COPD patients comparatively to control subjects and non-smoker COPD patients. With this data, we may suggest that severe COPD and smoking may enhance oxidative stress. The etiologic factors in COPD may increase oxidative stress not only through increasing oxidants, but also decreasing antioxidant capacity.<sup>1</sup> The findings obtained by Rahman et al<sup>2</sup> confirmed decreased antioxidant capacity in smokers and patients with COPD.<sup>2</sup> The antioxidant systems include enzymes (superoxide dismutases, catalase, glutathione peroxidase), proteins (albumin, ceruloplasmin, bilirubin, ferritin) and small molecules (ascorbic acid, reduced glutathione, alfa tocopherol, beta carotene, ubiquinol 10, methionine, and uric acid).<sup>2</sup> Paraonase is an enzyme which may have an antioxidant capacity in lung tissue, may be added to these antioxidant enzymes. Paraonase mainly localizes in Clara cells, endothelial cells and type 1 pneumocytes.<sup>7,9,10,22</sup> In their studies, James et al<sup>23</sup> showed in cardiovascular patients that smoking, which enhanced oxidative stress, also caused a significant decrease in activities of PON1. Boemi et al<sup>22</sup> found that smoking was associated with reduced activities of paraonase, in diabetics.<sup>22</sup> Several studies showed that smoking increased susceptibility of LDL to oxidative stress and that there were higher levels of oxidized LDL in smokers.<sup>23,24</sup> We found that serum activities of PON1 in COPD patients and in smokers with COPD were lower than normal subjects and non-smokers with COPD.

In conclusion, we suggest that smoking affects plasma trace element concentrations either directly or indirectly, and may contribute a decrease in antioxidant capacity as well as an increase in oxidant capacity.

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