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Clinical Problems

Angiotensin Converting Enzyme and D-Dimer Levels in Carbon Monoxide Intoxications

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Abstract

Objectives: Carbon monoxide (CO) inhalation injury is a complex processes and it is still dangerous. The aim of this study was to determine if there is any relation between elevation of biochemical markers obtained in carbon monoxide poisoning.

Patients: A total of 40 cases with CO intoxication that composed of 19 male and 21 female. Thirty healthy volunters were included to study as a control group. The patients were evaluated in two subgroups, group 1 = mild-moderate CO intoxication (26.33±10.96%) (n=25), group 2 = severe CO intoxication (52.21±8.29 %) (n=16).

Measurements and Results: Serum ACE activity, plasma fibrinogen and D-Dimer were measured. The mean angiotensin converting enzyme (ACE) value was significantly higher in group 2 (53.31±8.21 U/L) than both in group 1 (19.71±15.10 U/L) and the controls (17.4±7.88 U/L) ($p<0.05$). The level of D-Dimer in both group 1 (265.8±135.21) and group 2 (235.1±132.6) were significantly higher than that of the control (112.93±17.2) ($p<0.05$). A significant positive correlation was found between the CO and the ACE values ($r= 0.740, p<0.05$).

Conclusion: The interaction between CO and the ACE values suggested that secretion of ACE in response to hypoxia of the lung tissue increases as the CO increases, and D-dimer values were increased in all patients independent from the severity of the CO intoxication.

Key words: Carbon monoxide intoxication , ACE, Fibrinogen, D-dimer

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INTRODUCTION

Carbon monoxide is a colorless, odorless, tasteless gas that is a product of incomplete combustion of carbonaceous material. Common exogenous sources of CO include cigarette smoke, gasoline engines, and improper venting of fuel-burning space heaters [1]. Carbon monoxide intoxication is common in Turkey, especially in the winter season, because of the fuel burning equipments commonly used.

Carbon monoxide combines tightly with the heme Fe+2 of hemoglobin to form carboxyhemoglobin. The binding affinity of hemoglobin for CO is about 250 times greater than it is for oxygen. Carbon monoxide not only decreases the oxygen content of blood but also decreases oxygen availability to tissues, thereby produces a greater degree of tissue hypoxia than an equivalent reduction in oxyhemoglobin due to hypoxia alone [2]. However, other mechanisms that not related to tissue hypoxia have also been described as being involved in the harmful effects of CO poisoning. Carbon monoxide may bind to mitochondrial cytochrome c oxidase, which inhibits cellular respiration and aerobic adenosine triphosphate synthesis [3]. The disturbance in mitochondrial electron transport also causes generation of oxidative stress [4]. The toxic effects of CO are a result of a combination of tissue hypoxia and direct CO mediated damage at a cellular level.

In the pulmonary vascular bed angiotensin I converting enzyme (ACE) is localized in the caveolae intracellulares of the lung endothelial cells and communicated with the vascular lumen [5]. ACE is known to be a protein that has a potent enthothelial proteinase inhibitor of the lung. Abnormal serum ACE activity has been reported in various human lung disorders and in laboratory animals with acute lung injuries [6].

The fibrinolytic activity initiate by tissue and urokinase plasminogen activators both of which convert plasminogen into plasmin. Plasminogen activator inhibitor-1 (PAI-1), the principle inhibitor of the plasminogen system, irreversibly inactivates both tissue and urokinase plasminogen activators. D-dimer is a product that results from the breakdown of fibrin by plasmin and can serve as a marker for fibrinolytic system activity. It has been suggested that fibrin and its degradation products could potentiate the inflammation by modulating chemotaxis and endothelial cell injury [7].

There are several studies that reports significant increase in both ACE and D-dimer in several types of lung diseases but the association between the CO intoxication, coagulation products and ACE has not been explored previously [8-11]. The aim of this study was to evaluate changes in the serum ACE and D-dimer levels in hypoxia caused by high levels of carbon monoxide.

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MATERIALS AND METHODS

Patients who had been admitted to emergency service with the history of using fuel burning space and water heaters at home were enrolled in the study from 2006 to 2007. A total of 40 cases with CO intoxication that composed of 19 Male, and 21 Female (the mean age was 42.13 ± 15.21 years) were included into the study. The blood specimens of 30 healthy volunteers were studied as control group. The mean age was 30 ± 12 years (15 female and 15 male). Most of the patients were admitted to Emergency Service between 24:00 to 08:00 hours. None of them had history of allergy, smoke, liver disease or diabetes mellitus. One patient had a history of antihypertensive drug use (perindopril = coversyl) which had an inhibitory effect on ACE, therefore we excluded this patient from our groups but the findings of this patient were included. The patients who represented full conscious state were included into the study after getting their informed consent. The patients at unconscious state were included also after getting informed consent from their family. After the recovery, we obtained consent from themselves too. For comparison, thirty age and sex matched donors served as a control group from persons who visited our hospital for medical checkups and had no history or clinical evidence of any systemic or lung disease. The subjects who smoked or had systemic infections at the time of the study were also excluded.

All blood samples were drawn from the patients when they were admitted to emergency service. The arterial blood specimens collected into enjector included heparine and kept on ice for measuring arterial blood gases with Co-oximetry (Chiron diag. Rapidlab-865) and immediately analyzed near the patient. The plasma specimens were obtained by collecting bloods to the tubes containing sodium citrate for measuring both D-dimer and fibrinogen. ACE was assayed in Synchron CX7 (Beckman-Coulter CA, USA) and the principle of the method was based on the hydrolyze of furylacrylophenylalanylglycylglycine (FABPGG) to furylacrylophenylalanine and glycylglycine. Hydrolysis of FAPGG results a decrease in absorbance at 340 nm. The rate of decrease in absorbance is directly proportional to ACE activity in the sample (Sigma-Aldrich Diag. Taufkirchen, Germany). The reference range of ACE indicated by the manufacturer was 5-33 U/L. The assay kit for D-dimer was based on a latex-enhanced turbidymetric test for the quantitative determination of cross-linked fibrin degradation products in human plasma (D dimer PLUS reagent, Dade-Behring Inc. Newark, DE,USA). The assay kit for fibrinogen was based on the modified Clauss method. Citrated plasma is brought to coagulate with a large excess of thrombin. The coagulation time depends largely on the fibrinogen content of the specimens (Dade-Behring Inc. Newark, DE,USA). The reference range of the kit of D-dimer and the kit of Fibrinogen was 50- 192 μg/L and 1.8- 3.6 g/L, respectively.

Results were calculated as mean ± standard deviation for all parameters. The difference between the mean values of the patients that have normal distribution and the controls were detected by the One Way Analysis Of Variance (ANOVA) test. The values that have significant difference were applied Benforroni for Posthoc test. The difference between the mean values of the patients that have not normal distribution and the controls were detected by the Kruskal-Wallis test. The correlations between the CO values and the other parameters were calculated by the Pearson Correlation analysis. All statistical analysis were made with SPSS 11.0 program, defining statistical significance as $P<0.05$.

RESULTS

When the patients were evaluated with respect to the symptoms; the patients those had only headache and vertigo (n=16); the patients those had nausea and vomiting (n= 9) and the patients those had unconsciousness (n=15) were classified as showing mild, moderate and severe symptoms, respectively. The mean value of the CO in these three subgroups was 26.56±11.68 %; 26.10±10.24 % and 52.21±8.29 % respectively. The mean CO values of the patients who had the symptoms of headache and vertigo (n=16); and the noise and vomiting (n= 9) were very similar. Therefore, we combined the patients showing mild to moderate symptoms as group one. The mean CO value of group one was 26.33±10.96 %. The mean CO value of group two, those had a severe CO intoxication and had the

Table 1. The relationship between the mean value of parameters in controls and the patients

Parameters	Group 1 (n=25)	Group 2 (n=15)	CONTROL (n=30)
CO (%)	26.33± 10.96*	52.21 ± 8.29*	1.08 ± 0.22
PH	7.44 ± 0.056	7.35 ±0.08	7.42 ± 0,027
pCO2 (torr)	32.51 ± 5.11	43.21 ± 6.28	38.01 ± 3.21
ACE (U/L)	19.71±15.10	53.31±8.21*,**	17.4 ± 7.88
D-dimer(μg/L)	265.8±135.21*	235.1±132.6*	112.93 ± 17.2
Fibrinogen (g/L)	3.55±1.02*,**	2.82±0.11	2.63 ± 0.35

Group 1: The patients who have a mild to moderate CO intoxication
Group 2: The patients who have severe CO intoxication
*significant difference between the values of the controls and the patients ($p< 0.05$)
**significant difference between the values of group 1 and group 2 ($p< 0.05$)

symptom of unconsciousness (n= 15), was 52.21±8.29 %. None of the patients was lost to follow-up.

The mean ± SD values of the pH, pCO2, ACE, D-dimer and fibrinogen of the patients and the controls have been shown in Table I. As a result, the mean ACE value was significantly higher in group two than both in group one and the control group. Furthermore, a significant correlation was detected between the CO and the ACE values ($r= 0.740$, $p<0.05$). One CO poisoning patient (the value of CO= 38 %) who used an ACE inhibitor have ACE level lower than the detection limit (3.4 U/L). The level of fibrinogen was significantly increased in group one compared to both group two and the control group. In addition to these findings, the level of D-dimer in both group one and group two was significantly higher than the control group.

DISCUSSION

The results of this study showed that there was an enhanced serum ACE activity in severe CO intoxication and correlation was detected between the CO and the ACE values. D-dimer values were increased in all patients independent from the severity of the CO intoxication.

ACE is known to be a protein on the surface of the cell that has a potent enthothelial proteinase inhibitor of the lung. Abnormal serum ACE activity has been reported in various human lung disorders and in laboratory animals with acute lung injuries [6]. Therefore the secretion of ACE might be increased in order to prevent destructive activity of the proteinases in the hypoxic area of the lungs.

Integrity of the endothelial cell membrane is necessary for modulating changes in oxygen tension, and capable of initiating rapid changes in onset of the injury. Hypoxia results in increased cell ACE activity compared with cells exposed to normoxia [12]. Hypoxia induced alterations in lung and kidney ACE of the rat were reversible after return to a normoxic environment [13]. According to our results, ACE levels increased only in severe CO intoxication and this enhancement not only due to direct toxic effect of alveolar CO on endothelial cell, it may also be due to hypoxia.

Our another interesting finding was that the value of ACE found as "lower than detection limit" in the patient who used an ACE inhibitor. Only one example is not enough to reach definitive decision and further study is needed to examine the relationship between CO poisoning and ACE inhibitor use. Thus, it is also necessary to compare the patients with respect to use of ACE inhibitors.

PAI-1-deficient mice, which have a mild hyperfibrinolytic state, demonstrate reduced fibrin accumulation in response to in vivo hypoxic exposure compared with controls [14]. Under hypoxic conditions PAI-1 activity is significantly reduced in bovine lung endothelial cells [15]. Prior studies in humans in which modest levels of hypoxia caused rise in fibrin degradation products demonstrate activation of the fibrinolytic system [16]. Carbon monoxide inhibited the PAI-1 mRNA levels in mononuclear phagocytes, which reduced accrual of micro vascular fibrin [17]. Taken together the data from these prior experiments, we suggest the enhancement of D-dimer levels in CO intoxication might be due to PAI-1 inhibition. High plasma levels of D-dimer in CO poisoning may reflect the activation or injury of the endothelium associated with the activation of the coagulation-fibrinolysis system.

In conclusion, we have found elevation in ACE and D-dimer levels in CO poisoned patients. Further study is needed to determine whether these elevations can be used as biomarkers of severity of CO poisoning and as mechanistic determinants of CO pathophysiology.

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