Original Article

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Evaluation of Plasma Total Thiol Levels in Patients with Carbon Monoxide Poisoning in the Emergency Department

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Abstract

Objective: The study aimed to measure the levels of total thiol, an antioxidant parameter, in patients who presented to the emergency department (ED) with carbon monoxide (CO) poisoning under normobaric oxygen therapy (NBOT), evaluate the time-dependent changes in total thiol levels within the first 6 hours, and examine the course of antioxidants in CO poisoning.

Materials and Method: The study population consisted of 85 patients diagnosed with CO poisoning in the ED and 50 volunteers. Total thiol level was measured in the study group at the admission (T0), first (T1) and sixth hour (T6). Carboxyhemoglobin levels, cardiac markers, electrocardiography, and routinely requested tests were investigated in patients with poisoning. The total thiol level was measured in the volunteer group. Data analysis was performed with SPSS 16.0.

Results: There was a significant decrease in total thiol mean levels between T0 and T1 ($p < 0.01$), a significant increase between T1 and T6 ($p < 0.01$), and a significant increase between T0 and T6 ($p < 0.01$). No significant difference was found for T6 total thiol mean level between the patient and the control group ($p > 0.05$).

Conclusions: The significant decrease in the total thiol mean value from T0 to T1 may suggest that the oxidative stress continues within the first hour, and the initiation of the significant increase in the total thiol level within T1 may indicate that the oxidative stress decreased with treatment. Six hours of NBOT protocol is sufficient for acute CO poisoning in patients not requiring HBOT.

Keywords: Carbon monoxide poisoning, oxidative stress, antioxidant, total thiol level

Introduction

Carbon monoxide (CO) is a gas produced by the incomplete combustion of carbonaceous compounds, and its poisoning is associated with significant morbidity and mortality due to early and late complications[1,2]. The clinical presentation of CO poisoning differs according to the amount of CO in the environment, exposure duration, the health status of the victim, and the individual's metabolism factors[3]. As a result of poisoning, almost all systems are affected, especially vital structures such as the central nervous system, cardiovascular system, and respiratory system. Mortality is substantially associated with the involvement of those systems[4].

Oxidative stress has a major role in the pathophysiology of CO poisoning. Oxidative stress occurs with the binding of CO to hemoglobin and myoglobin and also free oxygen radicals that are increased by decreased function of oxidative enzymes such as cytochrome oxidase, guanylate cyclase, and nitric oxide synthase[5].

Antioxidants target to prevent the effects of increasing free oxygen radicals. Many antioxidants, such as enzymes, proteins, minerals, vitamins, glutathione, and thiol have been defined[6]. Thiols are a component of sulfhydryl groups attached to carbon atoms[7]. Thiols, which are endogenous molecules, help aerobic cells maintain in a reducing state despite the oxidizing environment. Thiols with exceptional antioxidant action provide a protective effect against cell damage induced by free radicals[8].

The primary aim of the study was to determine the dynamic changes of serum total thiol levels in patients admitted to the emergency department (ED) with CO poisoning receiving normobaric oxygen therapy (NBOT) at the time of admission (T0), the first (T1) and sixth hour (T6).

Material and Methods

This prospective case-control study was conducted in Ankara Atatürk Training and Research Hospital ED, after the approval of the ethics committee (2011/05/55). The informed written consent forms were received from each participant.

The patients with CO poisoning who presented to the ED within the first 6 hours after exposure to CO gas and received

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only NBOT between May 30, 2011, and November 15, 2011, were included in the study (n = 85). Blood carboxyhemoglobin (COHb) levels above 5% in non-smokers and 10% in smokers were accepted as CO poisoning. The control group participants (n = 50) were selected among the volunteer hospital personnel with similar characteristics in terms of age and gender distribution without any chronic diseases. Patients with a history of coronary artery disease, cerebrovascular disease, peripheral artery disease, and acute mesenteric ischemia were excluded. Patients younger than 18 years of age and those who refuse to participate were not included in the study.

Venous blood was collected from the antecubital regions of the patients at the admission (T0), the first (T1), and sixth hour (T6) after admission. Complete blood count, glucose, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) levels, sodium (Na), potassium (K), and calcium (Ca), creatinine kinase (CK), creatine kinase-MB (CK-MB), troponin I, and venous blood gases were studied routinely from the initial samples for each patient. The blood samples for total thiol measurement were kept at room temperature for 30 minutes and then centrifuged at 3500 rpm for 5 minutes. After centrifugation, the blood taken into Eppendorf tubes was stored at -80°C. All samples were dissolved simultaneously for the analysis.

Total serum thiol concentration or sulfhydryl group was measured by the method described by Elmman and modified by Hu. 5,5'-dithiobis (2 nitrobenzoic acid) (DTNB) interacting with thiol forms a highly colored anion with a maximum peak at 412 nm[9]. Total serum thiol values were obtained by adapting this method to the biochemistry analyzer (SIEMENS, ADVIA 2400, Japan) in the biochemistry laboratory of our hospital.

Data analysis was performed with SPSS 16.0 (IBM Corporation, Armonk, NY, USA). The Kolmogorov-Smirnov test was used for the normality analysis of data distribution. Descriptive statistics of the continuous and discrete numerical variables were expressed as mean ± standard deviation (± SD) and categorical variables were shown as number (n) and percentage (%). The student t-test was used to compare the study and control groups' selected parameters. The paired-sample t-test was used for the time-dependent changes in total thiol levels. Correlations analysis was performed for the relationship between the laboratory results A p value smaller than 0.05 was considered statistically significant.

Results

Of the 85 patients in the study group, 38 (44.7%) were male, and 47 (55.3%) were female. The mean overall age was 35.58 ± 14.57 (18-75). Twenty participants (40%) of those in the control group were male, and 30 were female (60%). The mean age of the control group was 34.3 ± 11.82 (18-60). The patient and the control group were similar in terms of age and gender distributions (p >0.05).

In the study group, the most common symptoms at admission were headache (81.2%) and nausea (72.9%). Chest pain (angina) was detected in 7 patients, and cardiac markers were found positive in 2 of those. Cardiac markers were also high in 2 patients who did not have any symptoms related to angina pectoris. The heart rate was above 120 beats/min in only 3 out of 9 patients with palpitations. Electrocardiographic pathologic changes suggesting cardiac ischemia were not detected in any of the patients. The distribution of the patient's symptoms was presented in Table 1.

Table 1: Symptoms of patients.

Symptom	%
Headache	81.2
Nausea	72.9
Dizziness	47.1
Vomiting	34.1
Syncope	18.8
Palpitation	10.6
Dyspnea	9.4
Angina	7.1
Speech disorder	3.5
Abdominal pain	2.4

The mean value of COHb (%) levels among the patient group was 23.198 ± 7.327. The patients were exposed to CO for an average of 174.88 ± 138.01 minutes. The most common exposure conditions were CO leaking from the stove (54.1%) and the water heater (42.4%). The demographic characteristics of the patients were summarized in Table 2.

Table 2: The demographic characteristics of the patients.

Characteristic	
Age (y), mean ± SD	35.58 ± 14.57
Gender	
Female (%)	47 (55.3)
Male (%)	38 (44.7)
Cause of poisoning (n)	
Stove	46
Water heater	36
Other	3
CO exposure duration (min), mean ± SD	174.88 ± 138.01
Admission method to the emergency department	
With an ambulance	61
By their own means	24
Vital signs	
Systolic blood pressure (mmHg)	121.50 ± 22.40
Diastolic blood pressure (mmHg)	77.17 ± 15.71
Pulse (beat/min)	87.67 ± 17.71
Respiratory rate (/min)	17.95 ± 3.80
Emergency department disposition (n)	
Discharge	79
Hospitalization	5
Transfer	0
Exitus	1

Table 3: Mean total thiol values of study and control groups

Parameter	Group	Time (hour)	Mean±SD
Thiol (µmol/l)	Patient	0th	423.16±86.94 (µmol/l)
		1st	370.90±67.47 (µmol/l)
		6th	474.56±48.52(µmol/l)
	Control	0th	482.94±92.18 (µmol/l)

The mean thiol value of the patients participating in the study was 423.16±86.94 µmol/l at T0, 370.90 ± 67.47 at T1, and 474.56 ± 48.52 at T6. The thiol value of the control group was measured as 482.94 ± 92.18 µmol/l (Table 3). When the means of total thiol levels at T0, T1, and T6 of the patients were compared, a statistically significant difference was determined. The mean thiol values of the patients at three different times and the thiol values of the control group were compared separately, and a statistically significant difference was observed between T0 thiol and the control group (p = 0.003). Also, the mean thiol value T1 and the control group differed (p = 0.001). However, there was no statistically significant difference between the thiol levels at T6 and the control group ($p > 0.05$).

There was a positive correlation between blood COHb levels and lactate levels of the patients in the study group (p <0.001). Correlations among other laboratory parameters are presented in Table 4.

Discussion

The study showed that the total thiol levels measured in patients followed up with the diagnosis of CO poisoning decreased in the first hour, and the levels measured at the 6th hour under NBOT increased up to the levels observed in the control group.

In CO poisoning, one of the primary damage mechanisms develops as a result of the deterioration of the balance against antioxidant activity due to an increase in oxidant production and insufficient antioxidant defense mechanism. With prolonged poisoning, tissue damage progresses, and mortality occurs[10]. Among the antioxidants, thiol groups have the highest concentration. The main sources of thiols are cysteine, homocysteine, methionine, and reduced glutathione (GSH) amino acids. The antioxidant capacity can be predicted by measuring the total thiol level in the plasma[11,12]. In 2009, Zhang J et al. measured the time-dependent changes in antioxidant levels and lipid peroxidation in order to understand the pathophysiology of neuronal damage in rats with CO poisoning. Malondialdehyde (MDA) level was also investigated for the detection of lipid peroxidation. They created CO poisoning by injecting CO into rats intraperitoneally. Considering the measurements made in the serum of rats on the 0th, 1st, 3rd, 7th, 14th, and 21st days from tissue samples taken from the cerebral cortex and hippocampus, the levels of MDA, as a lipid peroxidation marker, increased significantly. Another study found that antioxidant enzyme activities and glutathione increased on the 1st day but gradually decreased towards the 3rd, 7th, 14th, and 21st days[13]. In a study measuring mitochondrial oxidative stress as a result of hypoxia caused by CO poisoning in the brain of rats, catalase activity was investigated. The authors found that the activity of catalase, an antioxidant enzyme, decreased between 60 and 120 minutes under oxygen therapy but increased later[14]. Our study showed that thiol as an antioxidant increased gradually from the 0th to the 1st hour with NBOT in patients diagnosed with CO poisoning. The thiol values at T6 decreased and approached the thiol values of the control group, which consisted of almost healthy individuals.

In a randomized study performed on a series of 60 CO poisoning patients admitted to the hospital within 3 years in Spain in 2011, mitochondrial damage under NBOT was investigated by measuring mitochondrial complex IV activity. They divided the patients into two groups, the first group was composed of severe poisoning (n = 35) cases with a COHb level above 20%, and the second group was composed of moderate poisoning cases with a COHb level below 20% (n = 25). Two sessions of hyperbaric oxygen therapy (HBOT) were administered to 14 severe poisoning cases and 1 session to 21. Meanwhile, HBOT was performed in 14 of the moderate poisoning cases and NBOT in 11 of them. Mitochondria complex 4 activity was demonstrated to be significantly decreased in all severe and moderate poisoning cases compared to the control group. However, a more prominent decrease was detected in the group receiving HBOT. MDA level as the lipid

Table 4: Correlations of the laboratory parameters measured from patients.

	Ck-MB	Troponin	Lactate	COHb	Base Gap	WBC	Plt	Glucose
Ck-MB		$p < 0.01$ $r = 0.765$	$p < 0.01$ $r = 0.333$			$p < 0.01$ $r = 0.283$	$p = 0.012$ $r = 0.271$	$p = 0.036$ $r = 0.228$
Troponin			$p < 0.001$ $r = 0.425$		$p < 0.001$ $r = -0.424$		$p = 0.021$ $r = 0.249$	$p < 0.001$ $r = 0.401$
Lactate				$p < 0.001$ $r = 0.548$	$p < 0.001$ $r = -0.851$			$p < 0.01$ $r = 0.344$
COHb					$p = 0.004$ $r = -0.306$			$p < 0.01$ $r = 0.344$
Base Gap								$p < 0.01$ $r = -0.727$
WBC							$p < 0.01$ $r = 0.439$	

peroxidation activity indicator was high. As a result of HBOT and NBOT, the MDA level decreased significantly. There was a negative correlation between lipid peroxidation activity and mitochondria complex IV enzyme activity. While there was a correlation between COHb level and symptoms, no correlation was determined between mitochondria complex IV enzyme activity and symptom relief. Neurological sequelae developed in 5 patients, and although the decrease in mitochondrial complex IV enzyme activity was abnormal in those patients, normal levels were obtained with HBOT. Therefore, it was emphasized that mitochondria complex IV enzyme activity could be a valuable marker for treatment efficacy. It also raised the question of whether antioxidant therapy might be needed to reverse mitochondrial damage[14]. In our study, we found that thiol levels increased in patients with CO poisoning receiving NBOT. Since we referred patients who had HBOT indication to the relevant center where they could receive this treatment, their 1st, and 6th-hour total thiol values could not be measured. Thus, a comparison of thiol values between HBOT and NBOT could not be performed.

In the study by Taşkıran et al., the authors aimed to determine the changes in oxidative stress parameters in mitochondria by creating a CO poisoning model in rats[15]. Lipid peroxidation, cytochrome oxidase enzyme activity, and glutathione levels were measured from mitochondria in brain tissue (cortex, corpus striatum, and hippocampus). They found that cytochrome oxidase enzyme activity decreased in different parts of the brain in both groups, although at different rates. They reported that the glutathione level decreased in all rats with CO poisoning. In our study, we found that the thiol value decreased in patients with CO poisoning and increased after the start of NBOT. To reveal the role of oxidative stress in the pathophysiology of CO poisoning, Kavaklı et al. examined 88 patients in the emergency department and measured total oxidant status (TOS) and COHb levels[16]. Compared to TOS levels of the control group consisting of 35 healthy individuals, TOS levels of the patients diagnosed with CO poisoning were significantly higher. They reported that TOS, oxidative stress index (OSI), and COHb values measured after NBOT decreased significantly. In addition, TAS (total antioxidant status) levels measured after the treatment were compared with the TAS levels of the control group. They found no significant difference between the groups. Our study indicated that oxidative stress increased in CO poisoning, but after the oxygen treatment initiation, antioxidants increased while oxidative stress decreased.

The present study has several limitations. Firstly, the study was conducted in a single center on 85 patients. Secondly, we could not detect changes in total thiol values in patients receiving HBOT since our institution didn't have this modality.

Conclusion

In conclusion, thiol level increased significantly towards the 6th hour, although it decreased during the first hour (T0-T1). Increased oxidative stress and decreased antioxidants are the main changes in the pathophysiology of CO poisoning. The sixth-hour total thiol level of the patients with CO poisoning under NBOT reached the levels of healthy individuals which may suggest 6 hours of NBOT protocol is sufficient for acute CO poisoning in patients not requiring HBOT.

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