ORIGINAL ARTICLE

Mucahit Gunaydin[1]
Suha Turkmen[2]
Yunus Karaca[3]
Ozgur Tatli[3]
Furkan Yildirim[4]
Buket Akcan Altinkaynak[5]
Abdulkadir Gunduz[6]

[1]Giresun University, Faculty of Medicine, Department of Emergency Medicine, Giresun, Turkey
[2]Acibadem University, Faculty of Medicine, Department of Emergency Medicine, İstanbul, Turkey
[3]Karadeniz Technical University, Faculty of Medicine, Department of Emergency Medicine, Trabzon, Turkey
[4]Antalya Training and Research Hospital, Department of Undersea and Hyperbaric Medicine, Antalya, Turkey
[5]Ardahan University, Faculty of Health, Department of Nursing, Ardahan, Turkey
[6]Karadeniz Technical University, Faculty of Medicine, Department of Emergency Medicine, Trabzon, Turkey

Corresponding Author:
Mucahit Gunaydin
Giresun University, Faculty of Medicine, Department of Emergency Medicine
28100 Giresun - TURKEY
Tel: +90 505 807 53 54
E-mail: mgunaydin@hotmail.com

Received: 28.07.2017
Acceptance: 07.09.2017
DOI: 10.18521/ktd.331426

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

The Diagnostic Value of Protein Carbonyl Levels in Acute Carbon Monoxide Intoxication

ABSTRACT

Objective: Carbon monoxide (CO) is the main cause of intoxication-related mortality and morbidity in developed countries. It is responsible for more than half of fatal intoxications in many countries. The purpose of this study was to determine the diagnostic value of protein carbonyl (PC), a good marker of oxidative stress, in association with oxidative stress resulting from hypoxia emerging in patients with acute CO intoxication.

Methods: Thirty-four patients diagnosed with acute CO intoxication at the Emergency Department and 38 healthy volunteers were included in the study. Patients' PC levels at time of admission and after treatment were compared with those of a control group.

Results: No statistically significant difference was observed among PC levels at time of admission in the patient and control groups (p =0.305, patient group 0.025 ± 0.01, control group 0.026 ± 0.01). A significant decrease was determined in post-treatment PC levels in the patient group compared to those at time of admission (p = 0.006, admission 0.025 ± 0.01, post-treatment 0.017 ± 0.008). No significant correlation was determined between patients' carboxyhemoglobin (CO-Hb) levels and PC levels at time of admission (Correlation coefficient = -0.006, p= 0.971).

Conclusions: We think that PC is not suitable for use as a biomarker in the acute period in patients with CO intoxication.

Keywords: Carbon monoxide poisoning, Oxidative stress, Protein carbonyl.

Akut Karbonmonoksit Zehirlenmesinde Protein Karbonil Seviyesinin Tanısal Değeri

ÖZET

Amaç: Karbonmonoksit (CO) gelişmiş ülkelerde zehirlenme ile ilişkili mortalite ve morbiditenin ana nedenidir ve birçok ülkede ölümcül zehirlenmelerin yarısından fazlasından sorumlu tutulmaktadır. Bu çalışmada akut CO zehirlenmeli hastalarda meydana gelen hipoksi nedeniyle ortaya çıkan oksidatif strese bağlı olarak, iyi bir oksidatif stres markerı olan protein karbonilin (PC) tanısal değerini belirlemek amaçlanmıştır.

Gereç ve Yöntem: Acil serviste CO zehirlenmesi tanısı alan 34 hasta ve sağlıklı 38 gönüllü çalışmaya alınmıştır. Hastaların başvuru anı ve tedavi sonrası PC seviyeleri, kontrol grubu ile karşılaştırılmıştır.

Bulgular: Başvuru anında ortalama PC seviyeleri arasında hasta grubu ve kontrol grubu arasında istatistiksel olarak anlamlı fark tespit edilmemiştir, p = 0.305 (hasta grubu 0.025 ± 0.01, kontrol grup 0.026 ± 0.01). Hasta grubunda tedavi sonrası PC seviyelerinde başvuru anına göre anlamlı bir düşüş tespit edilmiştir, p = 0.006 (başvuru 0.025 ± 0.01, tedavi sonrası 0.017 ± 0.008). Hastalarda başvuru anında CO-Hb seviyeleri ile PC seviyeleri arasında istatistiksel olarak anlamlı bir korelasyon tespit edilmemiştir (Corelation coefficient = -0.006, p= 0.971).

Sonuç: PC'nin CO zehirlenmeli hastalarda akut dönemde bir biyobelirteç olarak kullanılmasının uygun olmadığını düşünmekteyiz.

Anahtar Kelimeler: Karbonmonoksit Zehirlenmesi, Oksidatif Stres, Protein Karbonil.

INTRODUCTION

Background: Carbon monoxide (CO) intoxications continue to represent a major public health problem worldwide. CO intoxications due to heating with wood-coal stoves in winter, or more recently to natural gas, are common. CO is the main cause of intoxication-related mortality in the developed world and is implicated in more than half of fatal intoxications in many countries (1).

CO is odorless, tasteless and colorless. It binds to hemoglobin with an affinity 200 times greater than that of oxygen. A decrease in the oxygen transport capacity of hemoglobin results in hypoxia in tissue and vital organs such as the brain and heart (2). However, tissue hypoxia alone does not explain the toxic effects that result. CO results in injury through oxidative stress (OS) and inflammatory processes that follow a hypoxic period and cell breakdown. CO directly impairs aerobic metabolism by binding to and inhibiting cytochrome oxidase (similarly to the effect of cyanide). CO binds to cytochrome c oxidase in the brain, resulting in an impairment of ATP synthesis and an increase in reactive oxygen species (ROS). Inflammatory changes in acute CO intoxication include intravascular neutrophil activation due to reaction with platelets. This causes neutrophil degranulation and perivascular OS (3).

Carboxyhemoglobin (CO-Hb) is measured when CO intoxication is suspected. A high CO-Hb level indicates exposure to exogenous CO and supports diagnosis. Despite being useful for diagnosis, initial CO-Hb measurement is not a reliable means of estimating the severity of CO intoxication and its long-term outcomes (3,4).

With an increase in OS, macromolecules such as intracellular lipids, proteins and DNA are compromised and cell injury or cell death occur. The presence of oxidative injury can be determined by measuring these macromolecules that emerge as a result of oxidative injury, such as malondialdehyde (MDA), protein carbonyl (PC) and 8-hydroxyguanin (8-OHG), in body fluids and tissues using biochemical techniques (5). Protein oxidation occurs as a result of covalent modification of proteins with ROS or OS products (6). Oxidative modification of proteins by reactive species is involved in the etiology and progression of a series of diseases, such as Alzheimer's, amyotrophic lateral sclerosis, cataract formation, chronic renal failure, cystic fibrosis, diabetes, ischemia-reperfusion injury, Parkinson's disease, rheumatoid arthritis and sepsis (7). PC is a chemically stable molecule, a characteristic which is useful in identifying and storing the molecule. PC levels are determined earlier in the blood compared to other OS markers such as glutathione disulfide or MDA, a marker of lipid peroxidation, and remains high in the blood for at least 240 min. Due to this early formation and relative stability, PC is superior to other products of oxidation. PC is therefore recommended as a marker of OS (8,9).

Importance: Previous studies have reported high PC in blood in ischemic diseases such as acute myocardial infarction (MI) and non-ST MI and in ischemia-reperfusion injury and have shown that this can be used as a diagnostic marker in determining ischemia and/or ischemia-reperfusion injury (9-11). Studies have shown the levels of various OS parameters in acute CO intoxication, but none have been conducted with PC, which is known to be superior to other parameters in showing OS.

Goals of this investigation: The purpose of this study was therefore intended to investigate the hypothesis that there may be an increase in PC levels in association with OS resulting from hypoxia in patients with acute CO intoxication and the clinical significance of variation in PC levels.

MATERIAL AND METHODS

Study setting: This research was performed as a single center at the Training and Research Hospital Emergency Department and prospective clinical study. Following approval from the ethical committee, 34 patients aged 18 or over with diagnosis confirmed on the basis of blood CO-Hb or referred from other hospitals with a diagnosis of CO intoxication in the six-month period between October 2013 and April 2014 were included in the study.

Patients and methods: Patients with a history of acute ischemic disease, such as acute coronary syndrome, acute ischemic cerebrovascular disease, acute peripheral artery obstruction or acute mesenteric ischemia, of advanced liver and heart failure, referred from other centers with a diagnosis of CO intoxication but with CO-Hb levels below 5%, patients developing arrest, subjects refusing to take part and patients with deficient study data were excluded. A control group was established consisting of 38 healthy, non-smoking volunteers working in fields involving no chronic exposure to CO.

Demographic data, major symptoms, vital signs (heart rate, systolic and diastolic blood pressure, respiration rate and body temperature), cause of intoxication, length of exposure to CO and Glasgow Coma Score (GCS) were for all patients at time of admission were recorded onto study forms. ECGs were performed at time of admission, and ECG changes were recorded. In addition, CO-Hb and PC levels were measured from venous blood collected at time of admission and at the end of treatment.

All patients received 100% normobaric oxygen (NBO) as initial treatment. Patients with prolonged confusion, neurological findings, cardiovascular dysfunction or severe acidosis and pregnant subjects with CO-Hb levels above 25% were treated with hyperbaric oxygen (HBO).

Duration of NBO therapy was determined on the basis of periodic investigation of blood CO-Hb values, and treatment was stopped when blood CO-Hb levels decreased to < 5%. HBO therapy was applied in a single 2-hour session at2.5 atmospheric pressure.

Sample collection and measurement of PC levels: Blood specimens of approximately 5 cc were taken for PC measurement at time of admission and at the end of treatment and placed into separator biochemistry tubes while avoiding hemolysis as much as possible. Specimens were centrifuged at 4000 rpm for 10 min in a centrifuge device. One cubic centimeter of serum was placed into Eppendorf tubes and stored at -80 oC until the day of study. Blood specimens for PC measurement from the control group were collected only once.

At the end of the data collection process, all specimens were studied concurrently by a researcher blinded to the study data and patient groups. PC levels in the serum specimens were determined using a colorimetric assay kit in line with the manufacturer's recommendations (Cayman Chemical Company Cat.No:10005020, Lot No. 0463100). The absorbances of specimens were measured at a light wave of 370 nm on a VERSA (designed by Molecular Devices in California, USA) microplate reader. The results were expressed as nmol/mL.

Statistical analysis: SPSS (Statistical Package for Social Sciences for Windows v.16.0) software was used for statistical analysis. Data compatibility with normal distribution was assessed using the Kolmogorov-Smirnov test. Wilcoxon's test was used to compare patient group pre- and post-treatment PC values, and the Mann-Whitney U test to compare the control group with the patient group pre- and post-treatment PV values. Correlation between CO-Hb and PC was calculated using the Spearman correlation test. Significance was set at $p < 0.05$.

RESULTS

Thirty-eight patients were enrolled in the study, together with a control group of 38 healthy volunteers. Four patients with deficient data during the study were excluded. Thirty-one patients were treated with NBO therapy and three with HBO therapy. No mortality occurred in any patients, and all cases resolved without sequelae. Baseline demographic and clinical characteristics of the study population are shown in Table 1. A classification of patients' major symptoms according to CO-Hb levels is shown in Table 2.

Table 1. Baseline demographic and clinical characteristics of the study population

Variables	Control group (n=38)	CO poisoning group (n=34)
Age, median (min,max) (y)	33 (18-79)	35 (18-80)
Sex (M/F)	20 / 18	13 / 21
Hemodynamic charecters		
• SbP, median (min,max)	110 (90-140)	120 (70-180)
• DbP, median (min,max)	70 (60-80)	70 (40-100)
• Heart rate, median (min,max), (beats/min)	74 (62- 90)	92 (64-130)
• Respiratory rate, median (min, max), (breaths/min)	14 (12-18)	20 (16-30)
Symptoms, n (%)		
• Headache		16 (47.1)
• Syncope		11 (32.4)
• Vomiting		4 (11.8)
• Dizziness		2 (5.9)
• Weakness		1 (2.9)
Cause of exposure to CO poisoning, n (%)		
• Coal-fired stove		30 (88.2)
• Hot water boiler		4 (11.8)
Length of exposure to CO, mean ± SD (h)		4.96 ± 0.59
Glasgow Coma Score at admission, n(%)		
• 8		1 (2.9)
• 11		1 (2.9)
• 14		2 (5.9)
• 15		30 (88.2)
ECG findings at admission, n (%)		
• Normal		23 (67.6)
• Sinusal taschycardia		11 (32.4)
Treatment applied, n (%)		
• Normobaric oxygen treatment		31 (91.2)
• Hyperbaric oxygen treatment		3 (8.8)
Treatment time, mean ± SD (h)		5.35 ± 2.2

SbP, siystolic blood pressure; DbP, diastolic blood pressure

Table 2. Numbers of major symptoms in patients and their classification according to CO-Hb levels, n (%)

CO-Hb levels	Syncope	Headache	Vomiting	Diziness	Weakness
<10%	2 (5.9)				
10%-20%		8 (23.5)	2 (5.9)		1 (2.9)
20%-30%	2 (5.9)	6 (17.6)		1 (2.9)	
30%-40%	6 (17.6)	2 (5.9)	2 (5.9)	1 (2.9)	
>40%	1 (2.9)				

Patient and control group PC levels at admission and after treatment are shown in Table 3 and Figure 1. No statistically significant difference was determined in terms of mean PC values at admission between the patient and control groups ($p = 0.305$, patient group 0.025 ± 0.01, control group 0.026 ± 0.01). A significant decrease was observed in post-treatment PC levels compared to time of admission in the patient group ($p = 0.006$, admission 0.025 ± 0.01, post-treatment 0.017 ± 0.008).

Table 3. Measured PC levels of the patients at admission and after treatment and control groups

Time dependent PC levels	Groups		
	Control group (n=38)	CO poisoning group (n=34)	p value
Admission	0.026 ± 0.01	0.025 ± 0.01	0.305[a]
After treatment		0.017 ± 0.008	
p value		0.006[b]	

[a] p value between the patient and control groups PC levels at admission, [b] p value between patient groups PC levels at admission and after treatment

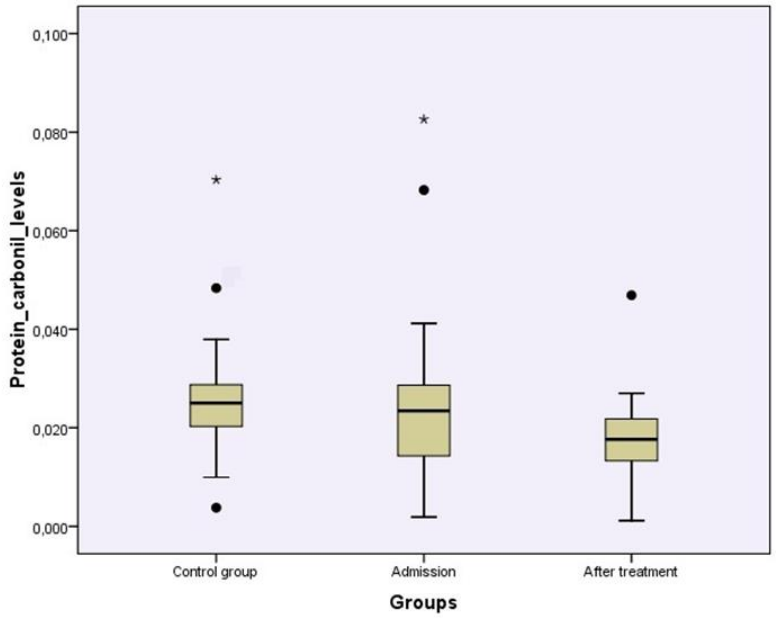

Figure 1. Measured PC levels of the control groups and the patients at the admission and after treatment.

Pre-treatment PC values were 0.025 ± 0.016 in the 31 patients receiving NBO therapy and 0.024 ± 0.001 in the 3 patients receiving HBO therapy due to increase of cardiac enzyme and low GCS. No statistically significant difference was determined between these two groups ($p = 0.879$)

No statistically significant correlation was observed between CO-Hb and PC levels at time of admission in patients, nor between GCS and PC values (Spearman correlation coefficient = -0.006 and -0.064, p= 0.971 and p= 0.718, respectively). At the same time, no difference was determined between blood PC levels when classification was performed on the basis of CO-Hb levels (Table 4).

Table 4. Mean PC levels among the study groups classified according to CO-Hb levels

CO-Hb groups	n	PC mean ± SD
≤10%	2	0.02 ± 0.00
10%-20%	11	0.02 ± 0.01
20%-30%	9	0.03 ± 0.03
30%-40%	11	0.02 ± 0.01
≥40%	1	0.01

DISCUSSION

This study investigated variation in levels of PC, in patients with acute CO intoxication, at time of admission to the emergency department and after treatment. While previous studies have investigated CO and OS markers, the principal distinguishing feature between this and other studies is that PC was investigated for the first time in the blood of patients with acute CO intoxication.

Our study findings showed that PC levels at time of admission in patients with acute CO intoxication were not statistically significantly higher than those of the control group. PC levels at time of admission in 31 patients receiving NBO therapy and 3 patients given HBO therapy did not increase significantly compared to the control group. No significant correlation was also determined in our study between CO-Hb levels and PC levels. In contrast to our studies findings, Kavakli et al. reported higher OS markers total oxidant status (TOS) and oxidative stress index (OSI) in patients with CO intoxication compared to a control group and determined a significant decrease in TOS and OSI following oxygen therapy (12). Wang et al. reported that CO-mediated neuron injury may be associated with an increase in lipid peroxidation and decreased antioxidative status. They reported time-dependent changes in lipid peroxidation and antioxidative status in serum, the cerebral cortex and hippocampus also included toxic effects of CO intoxication on neurons (13).

However, some studies in the literature support our findings with different OS markers. Garrabou et al. investigated the OS markers lipid peroxidation levels (MDA and 4 hydroxy-alkenal) in patients with CO intoxication. They reported that OS did not accompany CO intoxication and was of little use in showing the severity of such intoxication (14). Studies involving smoking, with a similar mechanism to that of acute CO intoxication, have reported that smoking does not lead to OS in the acute period and only produces OS in subjects with chronic dependence. This has been attributed to the need for a specific period of time to elapse for ROS products to accumulate or for antioxidant mechanisms to be compromised, for which reason OS does not appear in the acute period (15-18). The fact that PC levels did not increase in acute CO intoxication in our study may be due to the same cause.

Another finding from our study, PC levels decreased significantly after oxygen therapy compared to pre-treatment levels and the control group in this study. PC, which does not accompany CO intoxication in the acute period, may be used as an alternative to CO-Hb to observe the effectiveness of oxygen therapy. However, since our control group were healthy person they didn't oxygen theraphy, so we don't know what the final values of PC after oxygen treatment were in the control group. Therefore, we couldn't make comparison to PC levels after treatment of study group and control group.

The main limitation of this study is the small patient number. The fact that only 3 patients received HBO therapy prevented us from investigating more severe intoxication symptoms in the patient groups. Another limitation is that admission CO-Hb levels and PC levels may have been lower than expected since these patients were routinely given oxygen therapy by the 112 emergency teams during transportation to the emergency department.

CONCLUSION

We think that while PC is not suitable for use as a biomarker in the acute period in patients with CO intoxication, it may be used as an alternative to blood CO-Hb in observing the effectiveness of oxygen therapy in these patients. Wider-ranging studies with larger patient groups are now needed to confirm our findings.

REFERENCES

1. Hampson NB, Scott KL, Zmaeff JL. Carboxyhemoglobin measurement by hospitals: implications for the diagnosis of carbonmonoxide poisoning. J Emerg Med 2006;31:13-6
2. Dubrey SW, Chebab O, Ghonim S. Carbon monoxide poisoning: an ancient and frequent cause of accidental death. Br J Hosp Med (lond). 2015;76(3):159-62
3. Chiew AL, Buckley NA. Carbon monoxide poisoning in the 21st century. Critical Care 2014;18:221
4. Hampson NB, Hauff NM. Carboxyhemoglobin levels in carbon monoxide poisoning: do they correlate with the clinical picture? Am J Emerg Med 2008;26:665-9
5. Ozcan O, Erdal H, Cakırca G, et al. Oxidative stres and its impact on intracellular lipids, protein and DNA. J Clin İnvest 2015;6(3):331-6
6. Schacter E. Protein oxidative damage. Methods Enzymol. 2000;319:428-36
7. Kayalı R, Cakatay U. Protein oksidasyonunun ana mekanizmaları. Cerrahpasa J Med 2004;35:83-9
8. Dalle-Done I, Giustarini D, Colombo R, et al. Protein carbonylation in human diseases. Trends Mol Med. 2003; 9(4):169-76
9. Jamel MJ, Pereira Lde P, Mello NB, et al. Blood carbonyl protein measurement as a specific oxidative stres biomarker after intestinal reperfusion in rats. Acta Cir Bras. 2010;25(1):59-62
10. Maneewong K, Mekrungruangwong T, Luangaram S, et al. Combinatorial Determination of Ischemia Modified Albumin and Protein Carbonyl in the Diagnosis of Non ST-Elevation Myocardial Infarction. Ind J Clin Biochem. 2011;26(4):389-95

11. Pantke U, Volk T, Schumetzler M, et al. Oxidized proteins as a marker of oxidative stres during coronary heart surgery. Free Radic Biol Med. 1999;27(9-10):1080-6
12. Kavakli HS, Erel O, Delice O, et al. Oxidative stres increases in carbon monoxide poisoning patients. Hum Exp Toxicol. 2011;30(2):160-4
13. Wang P, Zeng T, Zhang CL, et al. Lipid peroxidation was involved in the memory impairment of carbonmonoxide-induced delayed neuron damage. Neurochem Res. 2009;34:1293-8
14. Garrabou G, Inoriza JM, Moren C, et al. Mitochondrial injury in human acute carbon monoxide poisoning: The effect of oxygen treatment. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2011;29(1):32-51
15. Alonso JR, Cardellach F, L´opez S, et al. Carbon monoxide specifically inhibits cytochrome c oxidase of human mitochondrial respiratory chain. Pharmacol Toxicol. 2003;93:142–6
16. Cardellach F, Alonso JR, L´opez S, et al. Effect of smoking cessation on mitochondrial respiratory chain function. J Toxicol Clin Toxicol. 2003;41:223–8
17. Mir´o O, Alonso JR, Jarreta D, et al. Smoking disturbs mitochondrial respiratory chain function and enhances lipid peroxidation on human circulating lymphocytes. Carcinogenesis. 1999;20:1331–6
18. Alonso JR, Cardellach F, Casademont J, et al. Reversible inhibition of mitochondrial complex IV activity in PBMC following acute smoking. Eur Respir J. 2004;23:214–8