

BNP shows myocardial injury earlier than Troponin-I in experimental carbon monoxide poisoning

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Abstract. – OBJECTIVE: In this study, our purpose was to determine whether plasma BNP level can be useful or not in determining the severity of myocardial injury formed by CO poisoning and to compare plasma BNP level with serum cTnI level.

MATERIALS AND METHODS: In the study, 46 female Wistar Albino rats were used. Rats were divided into four groups, one control group and three poisoning groups. The mixture of pure CO and air was injected for 60 minutes to provide 3000 ppm CO concentration. Blood samples of groups were collected to measure COHb, BNP and cTnI levels. Blood samples of poisoning groups were collected at the 1st, 6th and 12th hours after poisoning. After biochemical procedures, findings were analysed statistically and compared with each other.

RESULTS: Eight rats which died in poisoning groups were excluded and 38 rats were evaluated. BNP levels were high in all poisoning groups compared to control group and the difference between them was statistically significant ($p < 0.05$). cTnI levels were high in 6th and 12th hours poisoning groups compared to control and 1st hour group but only 12th hour group had statistically significant difference ($p < 0.05$). A statistically positive relation was established between BNP and cTnI levels in 6th and 12th hour groups ($R: 0.76 - p < 0.05 - n:38$).

CONCLUSIONS: It was found that BNP levels increased earlier than cTnI levels in acute severe CO poisoning. BNP levels of the cases which were determined to have increased cTnI levels showing myocardial injury increased as well. BNP can show myocardial injury and its severity in acute CO poisoning.

Key Words:

Carbon monoxide poisoning, BNP, Troponin, Carboxyhemoglobin, Myocardial injury.

Introduction

Carbon monoxide (CO) poisoning is one of the most frequent intoxications in the world, accounting for more than one-half of fatal poisonings reported in many countries and more than 50,000 Emergency Department (ED) visits per year in the US^{1,2}. Furthermore, it is the most common type of poisoning during winter months. The risk of death in patients over 65 years of age and men with alcohol intoxication is also higher^{3,4}.

The leading pathophysiological mechanism resides in the ability of CO to bind to hemoglobin molecules with high affinity, displacing oxygen and generating carboxyhemoglobin (COHb), which renders it virtually ineffective in delivering oxygen to the tissues⁵. The organs with the highest demand for oxygen, such as the brain and the heart, are more vulnerable to injury⁵⁻⁷. COHb formation alone is not able to completely explain the CO-related cardiac injury. Thus, several additional mechanisms were suggested such as interactions between myoglobin and cytochromes, free radical productions in ischemia-reperfusion injury, and disruption of CO's physical functions⁶⁻⁹.

The clinical spectrum of heart involvement in patients with CO poisoning is rather broad and typically encompasses cardiomyopathy, angina attack, myocardial infarction, arrhythmias, and heart failure up to myocardial stunning, cardiogenic shock, and sudden death^{1,10}. In autopsy samples, the pathological features of CO poisoning are variegated and may include scattered and punctiform necrotic areas, subendocardial hemorrhages in the left ventricle, degenerative involvement of papillary, and other muscles, as well as focal myocardial necrosis^{1,2,11}.

Monitoring markers including electrocardiogram (ECG), creatine kinase (CK), creatine kinase-MB (CK-MB), and troponins are recommended for the determination and follow-up of cardiac injury. Furthermore, echocardiography and coronary angiography are recommended for patients in whom signs of cardiac ischemia persist^{10,12}. Recent studies support the use of the new biochemical indicators such as B-type natriuretic peptide (BNP) and H-FABP in identifying the cardiotoxicity of CO poisonings at an early phase¹³⁻¹⁵. However, there are limited studies in the literature describing the relation between plasma BNP levels and cardiotoxicity in acute CO poisoning. The aim of this study was to investigate the plasma BNP levels in rats with acute CO poisoning, and to compare these results with troponin I levels.

Materials and Methods

Materials and Chemicals

A tube (BOS Ltd. Inc., UK) containing pure CO of 175 bar was used for the source of CO gas and CO gas detector (BW Technologies, Inc., Calgary, Alberta Canada) was used for the measurement of the CO of the environment. BNP (Rat BNP-32, Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) and serum cTnI (Rat cTnI, Life Diagnostics, Inc., West Chester, PA, USA) were used as the study chemical compounds.

Animals

Female Wistar Albino rats weighing 210-235 g were housed under standard laboratory conditions and were allowed free access to food and water. The investigation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-

23, revised 1996) and approval has been received from our institutional Animal Ethics Committee.

Experimental Design

The rats were divided into four groups as follows;

Group 1 (n = 10): No test procedure was performed. After blood samples had been taken by direct abdominal aorta puncture, the animals were sacrificed under anesthesia.

Group 2 (n = 12): The rats were exposed to CO gas. Evaluations were carried out at the 1st hour and blood samples were taken by direct abdominal aorta puncture. Then the animals were sacrificed under anesthesia.

Group 3 (n = 12): The rats were exposed to CO gas. Evaluations were carried out at the 6th hour and blood samples were taken by direct abdominal aorta puncture. Then the animals were sacrificed under anesthesia.

Group 4 (n = 12): The rats were exposed to CO gas. Evaluations were carried out at the 12th hour and blood samples were taken by direct abdominal aorta puncture. Then the animals were sacrificed under anesthesia.

CO Gas Exposing Protocol

Rats to be poisoned with CO were placed in an anesthesia chamber (dimensions 60 × 27 × 27 cm) made of transparent glass (Anesthesia chamber, Ejay International Inc., CA, USA). Twelve rats were placed into the chamber at every session. CO gas exposure was set to be 3000 ppm and this process continued for 60 minutes. Then rats were exposed to ambient air. Dying rats during or after the procedure were excluded from the study.

Surgical Procedures

All rats were anesthetized with 50 mg/kg intraperitoneal ketamine hydrochloride (Ketalar®), and blood samples were obtained by abdominal aorta puncture for blood, gas, and biochemical analysis. The rats were sacrificed by drawing blood from the abdominal aorta.

Biochemical Analysis

COHb levels were measured with a blood gas device (Rapidlab 865, Bayer Health Care, Tarrytown, NY, USA). The levels of serum cTnI and plasma BNP were measured using commercially available Enzyme-Linked Immunoabsorbent Assay (ELISA) kits. ELISA was performed according to the manufacturer's instructions.

Table I. The distribution of included rats according to groups and mortality rates.

	Alive		Dead		Total	
	n	%	n	%	n	%
Group 1	10	100	0	0	10	100
Group 2	10	83.3	2	16.7	12	100
Group 3	9	75	3	25	12	100
Group 4	9	75	3	25	12	100
Total	38	82.6	8	17.4	46	100

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences version 16.0 (SPSS for Windows 16.0, Inc, Chicago, IL, USA). All data were expressed as mean $\pm$ standard deviation ($\bar{X} \pm SD$). Kolmogorov-Smirnov test was used to evaluate whether all values conformed to a normal distribution. The importance of the difference between groups that conformed to the normal distribution was evaluated with one-way ANOVA test. Posthoc-Sheffe procedure was used to evaluate which group was different. Variables that did not conform to the normal distribution were tested with Kruskal-Wallis variance test. Bonferroni corrected Mann-Whitney U test was also applied as posthoc. The relationship between the variables was determined by the calculation of Spearman correlation quotient. A probability level of $p < 0.05$ was considered to be significant.

Results

The rats' distribution according to groups and mortality rates are presented in Table I. It was determined that 8 rats (17.4%) died, and that Groups 3 and 4 had the highest mortality rate. Furthermore, 38 alive rats (82.6%) were included in the study. The COHb, BNP, and cTnI levels of rats in the groups are shown in Table II. When

the data of COHb values of rats in the groups were evaluated, COHb levels of Group 2 and 3 were found to be statistically significant when compared with those of Group 1 ($p < 0.05$). However, the COHb values of Group 4 were observed to decrease to normal levels, and there was no statistically significant difference between them and Group 1 ($p > 0.05$). As compared to other groups, the highest statistically significant values were determined to belong to Group 2 ($p < 0.05$). Upon evaluation of the BNP levels of the rats in the groups, Group 1 was found to have the highest statistically significant BNP level among the four groups ($p < 0.05$). BNP levels were found to be higher in Group 3 and 4 than those in Group 2; but this difference was not statistically significant ($p > 0.05$). While the cTnI levels of Groups 1 and 2 were not found to differ significantly ($p > 0.05$), in Groups 3 and 4 it was significantly higher ($p < 0.05$). As the groups were compared among themselves, no statistically significant difference was found between Groups 2 and 3 ($p > 0.05$). However, the values of Group 4 were determined to be statistically significantly higher than Group 3 ($p < 0.05$).

The results of the evaluation of the correlation of COHb, BNP, and cTnI levels within the groups are presented in Figure 1. According to this, the values of COHb are observed to peak in the first hour of intoxication and decrease with

Table II. The COHb, BNP and cTnI levels of rats in the groups.

	Group 1 (n = 10) $\bar{X} \pm SD$	Group 2 (n = 10) $\bar{X} \pm SD$	Group 3 (n = 9) $\bar{X} \pm SD$	Group 4 (n = 9) $\bar{X} \pm SD$	p-value
COHb (%)	$0.83 \pm 0.34^{b,c}$	$65.5 \pm 4.1^{a,c,d}$	$20.7 \pm 5.9^{a,b,d}$	$1.06 \pm 0.26^{b,c}$	< 0.05
BNP	$5.7 \pm 3.3^{b,c,d}$	21.1 ± 11.7^a	31.4 ± 10.1^a	28.3 ± 9.5^a	< 0.05
cTnI	$0.07 \pm 0.02^{c,d}$	0.11 ± 0.05^d	$3.18 \pm 2.9^{a,d}$	$7.8 \pm 5.42^{a,b,c}$	< 0.05

a: Differing from Group 1; b: Differing from Group 2; c: Differing from Group 3; d: Differing from Group 4.

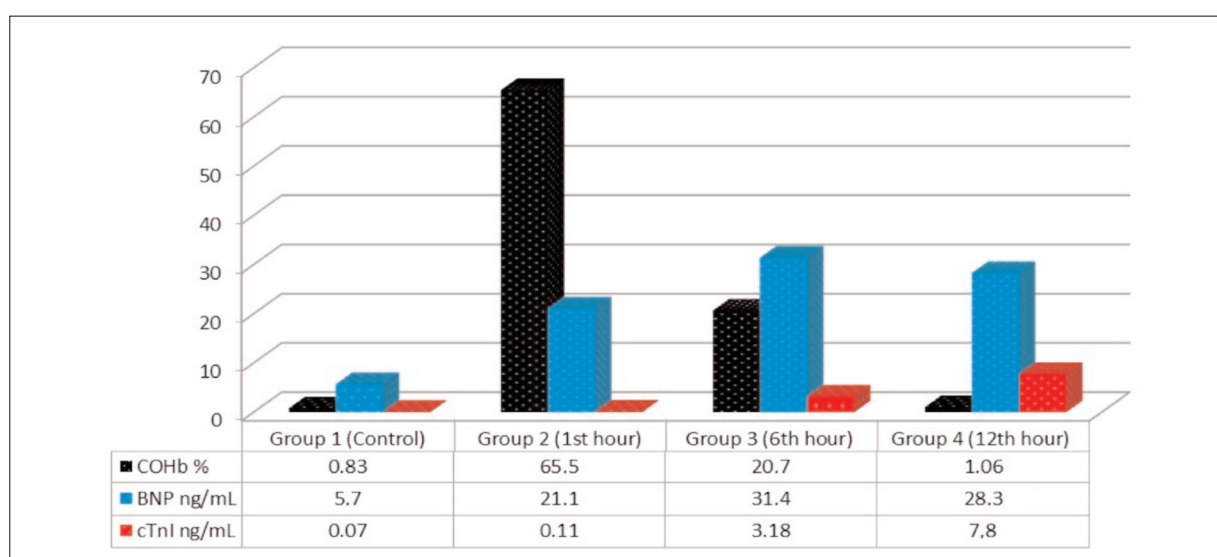


Figure 1. The correlation of COHb, BNP and cTnI levels among groups.

time as intoxication is cured. BNP levels are found to reach significant heights during the first hour of intoxication, and this increase was determined to peak in the 6th hour. A significant increase was observed in cTnI levels after 6 hours, and it was found to reach its peak at the 12th hour. In accordance with this, a direct relationship was observed between COHb and BNP levels. Furthermore, the increase and decrease of these two variables showed statistically positive significance ($R: 0.36 - p < 0.05 - n:38$). However, an inverse relationship was found between COHb and cTnI levels, the increase and decreases in the groups were found to be negative and statistically insignificant ($R: -0.02 - p > 0.05 - n: 38$). A direct relationship was observed between BNP and cTnI levels and the increase and decrease of these two variables showed statistically positive significance ($R: 0.76 - p < 0.05 - n: 38$).

Discussion

The clinical presentation of acute CO poisoning is variable, but in general, the severity of observed symptoms roughly correlates with the observed level of COHb. The patient is usually asymptomatic with levels less than 10%. As COHb increases above 20%, headache, dizziness, confusion, and nausea may develop. Coma and seizures resulting from cerebral edema are common with levels greater than 40%, and death is likely above 60%. Hypoxia is the main cause of

acute CO poisoning related mortality. Furthermore, mortality rates as high as 31% have been reported in large series, though in other surveys it was found to be only 1-2%¹⁶⁻¹⁸. In our study, the fact that COHb reached 65.5% and that there was a mortality rate of 17.4% at these levels support the literature.

Although cardiotoxicity is thought to be the main cause of mortality in CO poisoning, the early diagnosis of cardiac damage is often difficult. Many studies have been performed to date, but the positive contribution of ECG and CK, CK-MB in the diagnosis of cardiotoxicity has not been clinically proven to be useful. Actually, many factors may increase CK and CK-MB, which are known to be nonspecific markers¹². The assessment of cardiospecific troponins is now widely acknowledged as the mainstay for diagnosing a variety of myocardial injuries¹⁹. Their measurement is suited for defining the presence and severity of cardiac involvement after either ischemic or toxic injury, so that they would be particularly indicated in the context of CO poisoning, whereby both ischemia and direct myocardial toxicity may be involved⁵.

Satran et al¹² reviewed the cardiovascular manifestations of 230 consecutive patients treated for moderate to severe CO poisoning and found that only 16% of them had a normal ECG, whereas ECG abnormalities were present in as many as 30% of patients. Accordingly, significant elevation of CK-MB and troponin I were present in 35% of patients. Aslan et al²⁰

investigated 40 consecutive adult patients with CO poisoning. Significantly increased CK and CK-MB levels with normal troponin T were reported in 15% of cases, whereas substantially increased cTnT levels were only present in 1 case. Henry et al²¹ prospectively investigated 230 consecutive adult patients treated for moderate to severe CO poisoning. Myocardial injury, as established by a cTnI level ≥ 0.7 ng/mL or CK-MB level ≥ 5.0 ng/mL and/or diagnostic ECG changes occurred in 37% of patients. At a median follow-up of 7.6 years, as many as 54 deaths occurred (24%), 4 of which (2%) as a result of a combination of cardiac arrest and anoxic brain injury. Kalay et al¹⁰ evaluated cardiac structures and function in 20 patients with CO poisoning. cTnI was elevated in 30% of patients and was positively correlated with higher carboxyhemoglobin levels as well as CO exposure. cTnI levels were evaluated in our study and a significant increase was observed after the 6th hour, while it was found to reach its peak at the 12th hour. An inverse relationship was found between COHb and cTnI levels, the increase and decreases in the groups were found to be negative and statistically insignificant.

The natriuretic peptides, including atrial natriuretic peptide, BNP, and N-terminal proBNP (NT-proBNP) are a class of endogenous mediators that are secreted in response to any cause of myocardial stress^{22,23}. Davutoglu et al¹³ assessed plasma NT-proBNP levels in 15 healthy controls and 15 patients who were admitted with acute CO poisoning. Interestingly, no difference was observed between the plasma levels of NT-proBNP, as well as those of CK, CK-MB, and cTnT between cases and controls, whereas those of NT-proBNP were found to be increased in the intoxicated group. A highly significant, positive correlation was also found between the COHb and NT-proBNP levels. It was, thereby, concluded that natriuretic peptides may be used as surrogate biomarkers of cardiotoxicity and heart dysfunction in CO poisoning. They also concluded that measuring the plasma NT-proBNP levels may contribute to the early diagnosis of cardiotoxicity in patients with CO poisoning, together with the conventional methods and markers, such as echocardiography, CK, CK-MB, and ECG. Goetze et al²⁴ suggest that acute myocardial hypoxia results in a rapid increase in cardiac BNP gene transcription, release of proBNP peptide from the ventricular myocytes, and a significant rise in plasma NT-proBNP concentrations.

Our study supports the literature in that plasma BNP levels are found to increase in intoxication groups. Furthermore, this increase was found to occur rapidly following intoxication, as Goetze et al²⁴ suggested. Also, as in the study of Davutoglu et al¹³ the direct relationship we determined between COHb and BNP and the positive significance of the increase and decrease between these two variables are worthy of attention. In addition to those, the direct relationship between BNP and cTnI, as we determined in our study, and the statistically positive correlation between the increase and decrease between these two variables, are supportive of the literature and expected.

Conclusions

Plasma BNP levels increase more rapidly than cTnI following CO poisoning, just like COHb levels. Furthermore, this increase shows a positive correlation with cTnI with regards to revealing cardiac damage. This result shows that plasma BNP levels may be used for the early detection of cardiac injury occurring due to CO poisoning; however, further studies which support this conclusion are needed.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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