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The Impact of Carbon Monoxide Intoxication on Thiol/Disulfide Hemostasis

Özge Ebru Dağcı Varhan[1(ID)], Gülşah Çıkrıkçı Işık[2(ID)], Mustafa EKİCİ[3(ID)], Tuba Şafak[4(ID)], Şeref Kerem Çorbacıoğlu[5(ID)], Yunsur Çevik[5(ID)]

[1]İzmir Bornova Türkan Özilhan State Hospital, İzmir, Turkey.
[2]Atatürk Sanatoryum Training and Research Hospital, Department of Emergency Medicine, Ankara, Turkey
[3]Bitlis City Health Management Office, Bitlis, Turkey
[4]Ankara Etlik City Hospital, Department of Emergency Medicine, Ankara, Turkey
[5]Atatürk Sanatoryum Training and Research Hospital, Department of Emergency Medicine, Ankara, Turkey

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Abstract

Objective: Carbon monoxide (CO) poisoning is an oxidative stress factor. The aim of the study is to evaluate impact of CO intoxication on thiol/disulfide homeostasis (TDH), an important antioxidative system of the body.

Methods: This is a prospective study included 84 participants in each group. Blood samples were taken two time in study group (CO intoxication group), before and at the end of the 3rd hour of normobaric oxygen therapy and once in the control group. TDH parameters were studied with an automated assay developed by Erel et al. Statistical analysis done with SPSS program.

Results: Among thiol/disulfide homeostasis parameters, in study group native (sh) and total thiol (tt) levels in samples taken at the beginning of the oxygen treatment were significantly higher than sh and tt levels of the control group [Study group sh: 399.70 µmoll−1 (354.50-423.65), tt: 439.1 µmoll−1 (390.9-467.3) and control group sh: 362.95 µmoll−1 (321.95-401.25), tt: 396.1 µmoll−1 (358.5-435), p=0.01 and p<0.001 respectively]. There was no difference between the groups in term of other TDH parameters. TDH parameters were measured after 3-hour normobaric oxygen treatment, and it was shown sh and tt levels were significantly reduced after treatment.

Conclusion: Our study demonstrated that among TDH parameters native and total thiol levels were increasing in patients with CO poisoning and those levels were decreasing in time during normobaric oxygen treatment.

Key words: Carbon monoxide intoxication; Thiol/disulfide homeostasis; oxidative stress; antioxidant systems

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Address for correspondence/reprints:

Gülşah Çikrikçi Işik

Telephone Number: + 90 (505) 587 3436

E-mail: gulsah8676@gmail.com

INTRODUCTION

Carbon monoxide (CO) which is also known as “silent killer” is an odorless, colorless, nonirritant gas that is produced by incomplete combustion of carbon-containing components (1). Main mechanism of action is the binding of CO to hemoglobin with a higher affinity, that results with decreased oxygen presentation to tissues. CO also, directly effects electron transport systems in mitochondria and causes an oxidative stress (2). Those oxidative stress triggers many metabolic reactions in platelets, leukocytes, and endothelium and finally, CO intoxication results with production of free radicals, apoptosis, endothelial dysfunction, and lipid peroxidation (3).

Free oxygen radicals are removed by antioxidative systems in the body (4, 5). Thiol/disulfide hemostasis (TDH) is one of the important components of these antioxidative systems. Thiol which is an organic component that contains sulfhydryl group (-SH), exists in proteins such as albumin, cysteine, methionine (6). In case of oxidative stress, thiol groups oxidized and turn into reversible disulfide bridge, that reduced back to thiol groups again. Dynamic TDH is crucial for cellular signal mechanisms, antioxidant defense, detoxification, apoptosis, inflammation, and immune response (3).

The aim of this study is to evaluate changes in thiol/disulfide homeostasis during an important oxidative stress factor, CO poisoning.

METHODS

This is a prospective study, conducted in a training and research hospital with approval of the local ethics committee during six months period. Study included 84 patients diagnosed as carbon monoxide poisoning during their ED visit and 84 healthy volunteers as control group.

Study process

Diagnosis of CO intoxication done with measurement of carboxyhemoglobin (COHb) levels in venous blood samples of patients who had suspicious complaints for CO intoxication like headache, dizziness, etc. Patients who have COHb levels greater than 10% regardless of smoking habit, diagnosed as CO intoxication. Patients younger than 18, who did not consent to participate, who had malignancies or chronic inflammatory diseases, who were pregnant and who had trauma were excluded. After initial evaluation patients in the study group treated with normobaric oxygen. Demographic variables, vital sings and laboratory findings of the participants were recorded into the study forms.

Measurement of thiol/disulfide homeostasis

In the study group blood samples were taken two times, first at the beginning of oxygen treatment and second at the end of the 3rd hour of oxygen treatment; in the control group blood samples were taken only once. The participants’ blood samples were stored at –80°C after ten minutes of centrifuge at 3600 cycles. All samples were dissolved simultaneously and studied with

an automated assay developed by Erel et al. with a Roche Hitachi Cobas c501 automatic analyzer (6). By this method we measured the total thiol (tt), native thiol (sh), disulfide (ss), native thiol/total thiol % (sh/tt), disulfide/native thiol % (ss/sh) and disulfide/total thiol % (ss/tt) levels.

Statistical analyses

Statistical analyses were done with SPSS 16.0 (Chicago, IL, USA). Distribution of normality tested with Kolmogorov–Smirnov test and data did not fit normal distribution expressed as median and inter quartile range 25-75. Categorical variables were expressed as number and percentages. Comparison between independent groups were done with Mann-Whitney U test and between dependent groups with Wilcoxon singed-rank test. P value <0.05 considered statistically significant. ROC analyze was done to define cut off values. The value at which sensitivity, specificity, positive predictive and negative predictive values were all greatest, chosen as the best cut off value.

RESULTS

Results of 168 participants (84 in each group) were evaluated. Gender distribution and median age was similar between study and control groups. There was no difference in terms of vital parameters, hemogram values and biochemical parameters; despite lactate, blood urea nitrogen (BUN), glucose and aspartate aminotransferase (AST) which were significantly higher in the study group. Median level of COHb was %27.7 (18.6-32.9) in the study group. (Table 1).

As a result of PCR test, 66 (66%) of the patients were negative and 34 (34%) were positive. All of the patients who were found to be negative with the rapid antigen test were also found to be negative with the PCR test. Only 24 of the 34 PCR positive patients were also positive with the antigen test (Table 1).

Among thiol/disulfide homeostasis parameters, in study group native and total thiol levels in samples taken at the beginning of the oxygen treatment were significantly higher than the native and total thiol levels of the control group [Study group sh: 399.70 µmoll−1 (354.50-423.65), tt: 439.1 µmoll−1 (390.9-467.3) and control group sh: 362.95 µmoll−1 (321.95-401.25), tt: 396.1 µmoll−1 (358.5-435), p=0.01 and p<0.001 respectively]. There was no difference between the groups in term of other TDH parameters (ss, sh/tt, ss/sh and ss/tt ratios, p>0.05 for all circumstances). (Table 2).

In the study group TDH parameters were measured after 3 hour normobaric oxygen treatment and it was shown that native and total thiol levels were significantly reduced after treatment [At the beginning of the treatment sh: 399.7 µmoll−1 (354.5-423.6), tt: 439.1 µmoll−1 (390.9-467.3), at the end of the 3rd hour of oxygen treatment sh: 354.1 µmoll−1 (309.2-398.5), tt: 401.3 µmoll−1 (354.4-444.5), p<0.001 for both]. There was no change in other TDH parameters with treatment. (Table 2).

Table 1. Demographic characteristics, vital parameters and laboratory findings of study and control groups.

	Study group n=84	Control group n=84	P value
Demographics			
Gender: female/male	42/42	39/45	0.6
Age: median (IQR25-75)	36 (26-48)	37.5 (27-49)	0.6
Vitals			
Median (IQR25-75)	125(119-134)	130(121-138)	0.09
Systolic blood pressure	70(63-80)	73(64-80)	0.54
Diastolic blood pressure	80(72-97)	73 (64-80)	0.74
Heart rate	36.2(36-36.5)	36.1(36-36.5)	0.85
Temperature	96 (94-98)	97 (95-98)	0.09
Oxygen saturation			
Laboratory findings			
Median (IQR25-75)			
COHb (%)	27.7 (18.6-32.9)	0.1 (0-0.1)	<0.001
pH	7.41 (7,36-7,45)	7.41 (7.38-7.42)	0.9
Lactate (mmol/L)	1.9 (1.2-2.6)	1.2 (0.9-1.77)	<0.001
Hemoglobin (gr/ dl)	13.9 (12.7-15.3)	13.2 (12.1-14.8)	0.03
White blood cell (10-3/ µl)	9.07 (7.4-12.1)	8.8 (7.1-11.3)	0.5
Platelet (10-3/ µl)	233 (204-287)	256 (223-308)	0.03
Glucose (mmol/L)	106 (96-127)	100 (90-111)	0.007
AST (U/L)	20 (16-26)	17 (14-23)	0.03
ALT (U/L)	18 (13-24)	15 (11-22)	0.07
Creatinine (mg/dl)	0.78 (0.67-0.89)	0.72 (0.66-0.80)	0.16
BUN (mmol/L)	13 (11-16)	13 (10-15)	0.01
Troponin I (ng/ mL)	2.1 (0.6-5.1)	1.65 (0.8-2.6)	0.14

Table 2: Levels of thiol/disulfide parameters in control and study groups

TDH parameters Median (IQR25-75)	**Control group**	**Study group 1st measurement**	**Study group 2nd measurement**	**p1***	**p2****
Native thiol (µmoll−1)	362.95 (321.9-401.2)	399.7 (354.5-423.6)	354.1 (309.2-398.5)	0.01	<0.001
Disulfide (µmoll−1)	17.5 (10.9-23.1)	19.7 (15.7-23.02)	19.6 (15.5-24)	0.09	0.57
Total thiol (µmoll−1)	396.1 (358.5-435)	439.1 (390.9-467.3)	401.3 (354.4-444.5)	<0.001	<0.001
Native thiol/total thiol %	91.1 (87-94.3)	90.9 (89.6-92.04)	90.1 (88.1-92.3)	0.43	0.23
Disulfide/native thiol %	4.87 (3.02-7.27)	5.01 (4.33-5.78)	5.5 (4.1-6.8)	0.43	0.21
Disulfide/total thiol %	4.44 (2.83-6.48)	4.55 (3.97-5.19)	5 (3.8-6)	0.43	0.23

*p1: Comparison of thiol/disulfide parameters between control group and 1st measurement (beginning of the oxygen treatment) of the study group.

**p2: Comparison of thiol/disulfide parameters between 1st measurement (beginning of the oxygen treatment) and 2nd measurement (at the end of the 3rd hour of oxygen treatment) of the study group.

To evaluate clinical value of total and native thiol for diagnosis of CO poisoning, we made receiver-operating characteristic (ROC) analyze. Area under curve (AUC) was calculated as 0.65 for native thiol and 0.67 for total thiol. Best cut off value for native thiol was 383 µmoll−1 with 61% sensitivity and 61%specificity, 61% positive predictive value (PPV) and %61 negative predictive value (NPV); for total thiol it was 425 µmoll−1 with 62% sensitivity and 67% specificity, 65%PPV and 64% NPV. (Figure 1)

Figure 1: ROC curve for native and total thiol levels for diagnosis of CO poisoning.

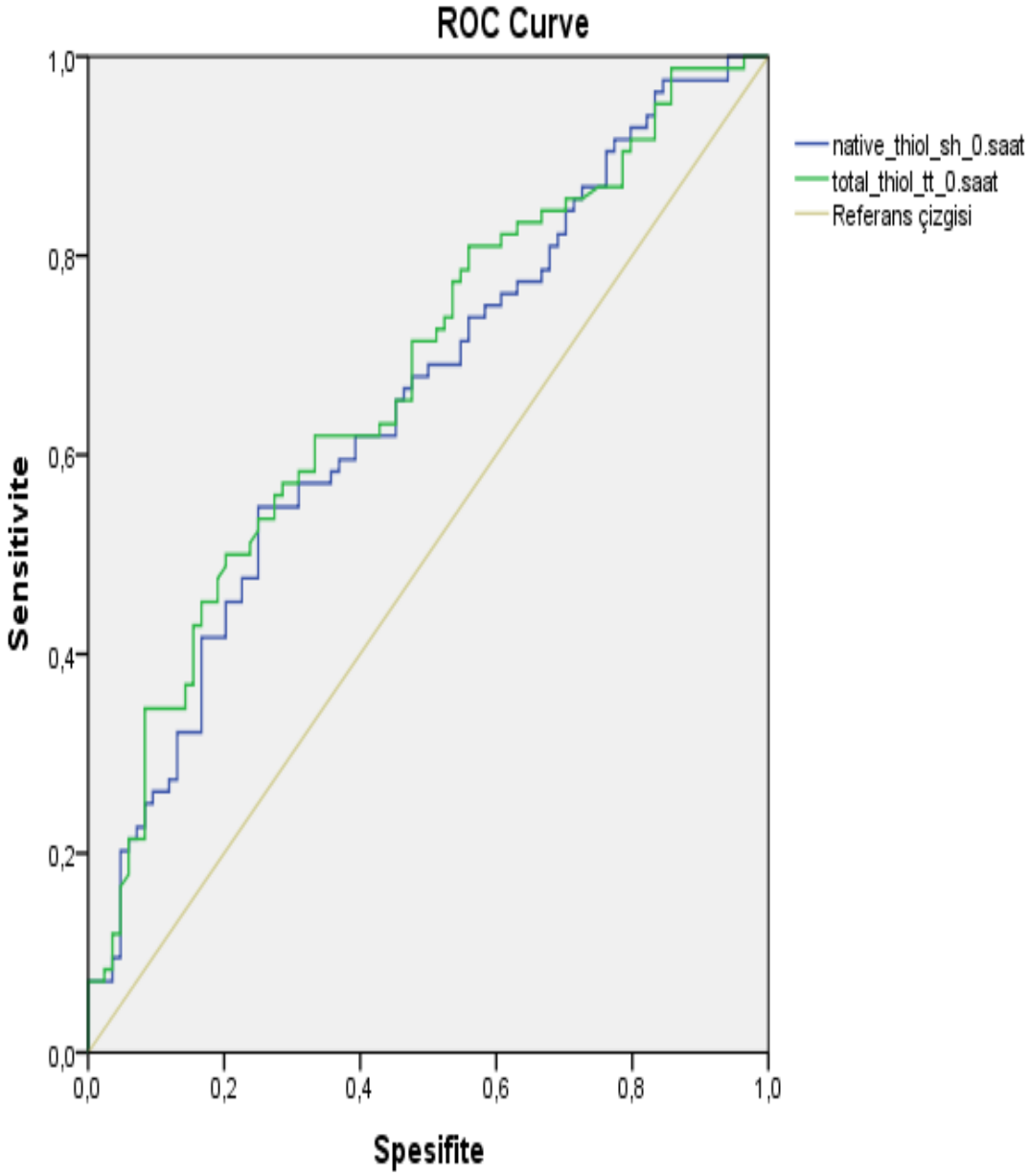

Figure 1. *AUC for native thiol, 0.65; for total thiol, 0.67. P values are 0.001 and <0.001 respectively*.

DISCUSSION

Our study, at which we analyzed the impact of CO intoxication on thiol/disulfide homeostasis, demonstrated that among TDH parameters, only native thiol and total thiol levels were changed with CO intoxication. Despite there was a statistically significant increase at native and total thiol levels during CO intoxication, ROC analyzed showed low AUC values, that means TDH parameters are not useful for differential diagnosis of CO poisoning. Our data also showed, among CO intoxicated patients, native and total thiol levels were decreasing in time during normobaric oxygen therapy.

Main mechanism of CO toxicity defined through hemoglobin, myoglobin, cytochrome oxidase, and cytochrome P450-dependent mechanisms; that higher affinity of those molecules to CO than oxygen leading to tissue hypoxia, myocardial ischemia, and disruption of cellular respiration (7). On the other hand, effect of CO on intracellular targets has not well understood yet but, reason of the cellular damage thought to be the result of oxidative stress. Studies demonstrated that, delayed encephalopathy at CO intoxicated patients is a consequence of lipid peroxidation triggered by oxidative stress (8). Kavaklı et al. studied total oxidant, total antioxidant status and oxidative stress index in their study conducted with CO intoxicated patients and showed that total oxidant status level and oxidative stress index were significantly higher at CO poisoned group than the control group (9,10). Also, among CO poisoned group those parameters were higher at the beginning of the oxygen therapy, than the levels after the treatment.

Free radicals are produced during normal physiologic process of the body and oxidative effect of those molecules neutralized by antioxidant capacity of the cells; if oxidative stress overwhelms the antioxidative defense, cellular damage occur (11,12). One of the important antioxidant mechanisms of the body is dynamic thiol/disulfide homeostasis. Until now, TDH parameters were studied in several different pathologies, such as endocrine disorders, cardiac pathologies, neurologic diseases, gastrointestinal disorders etc., to elucidate the pathologic mechanism, as a predictor for diagnosis or as a prognostic marker (13). Also, there were studies about TDH parameters in CO intoxicated patients, but they have some conflicting results.

Ergin et al. demonstrated in their study that native thiol and total thiol levels were significantly lower and disulfide was significantly higher in CO intoxicated patients comparing to control group (shCO: 344.29 ± 62.29, shControl: 475 ± 49.01, ttCO: 385.71 ± 66.92, ttControl: 507.87 ± 50.54, ssCO: 20.7 ± 5.03, ssControl: 16.43 ± 3.97 μmol/L, $p<0.001$, $p<0.001$ and $p=0.001$ respectively) (3). Those results were completely different from our results, we found higher native and total thiol

levels in CO intoxicated group and no significant difference in disulfide levels between the groups. The reason of this different results might because of the small sample size and timing of sample intake at Ergin's study. Ergin et al. also studied other oxidant and antioxidant parameters in their study and showed reduced total antioxidant response, paraoxonase and arylesterase levels in CO intoxicated group, with significantly increased total oxidant status and ceruloplasmin levels.

On the other hand, İşler et al., similar with our results demonstrated that native and total thiol levels were significantly higher in CO intoxicated patients and disulfide levels were similar between the groups (shCO: 382.8 ± 106.1, shControl: 330.9 ± 101.7, ttCO: 416.1 ± 98,6, ttControl: 371.0 ± 98.0, ssCO: 16.50 ± 8.15, ssControl: 15.57 ± 7.30 µmol/L, p=0.006, p=0.006, p>0.05 respectively) (14). However, in this study differently from our results native and total thiol levels were increased more after normobaric oxygen therapy. İşler et al. also analyzed the oxidative stress parameters between the groups who received normobaric or hyperbaric oxygen treatment and there was no difference between the groups.

In another study comparing TDH parameters among CO intoxicated patients receiving normobaric or hyperbaric oxygen therapy, Bağcı et al. showed a decrease in native and total thiol levels after treatment with hyperbaric oxygen therapy; there was no difference in TDH parameters before and after the treatment with normobaric oxygen (15). In this study indication of hyperbaric oxygen was having COHb level greater than %15. In our study group median COHb level was 27.7%. Therefore, we might think similar oxidative stress levels in our study group and patients received hyperbaric therapy at that study and our TDH parameter changes were similar with Bağcı et al.'s study. So, role of thiols in antioxidant systems are very complicated and despite knowing thiol disulfide homeostasis' crucial role in redox systems, it is still not known clearly how the balance is achieved in this mechanism (16).

This study has some limitations. First, we did not analyze other oxidative stress markers and total antioxidant status of the patients, which might be helpful to interpret the oxidative status better. Second, we did not evaluate the patients who transferred for hyperbaric oxygen therapy later. It should be better to study TDH parameters before and after hyperbaric oxygen therapy to understand effects of this treatment on oxidative stress.

CONCLUSION

Our study demonstrated that among TDH parameters native and total thiol levels were increasing in patients with CO poisoning and those levels were decreasing in time during normobaric oxygen treatment. There are conflicting results in the literature and to clearly understand how the balance in TDH is

achieved, more comprehensive studies are needed.

Ethical Approval: Ethical approval for this study was obtained from Kecioren Training and Research Hospital Ethics Committee (No: 2012-aKAEK-15).

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Informed consent: Written informed consent was not necessary, the present study retrospective and no specific patient data has been included in the manuscript.

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