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Detection of neutrophil–lymphocyte ratio as a serum marker associated with inflammations by acute carbon monoxide poisoning

Mustafa Karabacak[1], Kenan Ahmet Turkdogan[2*], Abuzer Coskun[3], Orhan Akpinar[4], Ali Duman[2], Mücahit Kapci[2], Sevki Hakan Eren[3], Pınar Karabacak[5]

[1]*Isparta State Hospital, Department of Cardiology, Isparta, Turkey*

[2]*Adnan Menderes University, Department of Emergency Medicine, Aydin, Turkey*

[3]*Cumhuriyet University, Department of Emergency Medicine, Sivas, Turkey*

[4]*Suleyman Demirel University, Department of Microbiology, Faculty of Dentistry, Isparta, Turkey*

[5]*Suleyman Demirel University, Department of Anesthesia and Reanimation, Medical Faculty, Isparta, Turkey*

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ABSTRACT

Objective: To investigate neutrophil–lymphocyte ratio (NLR), which is an indicator of systemic inflammation, in patients with carbon monoxide (CO) poisoning.
Methods: We included 528 patients (275 women) who presented with a diagnosis of CO poisoning between June 2009 and March 2014. Control group was composed of 54 patients (24 women). Platelet count and mean platelet volume level were significantly higher in the CO poisoning group.
Results: White blood cell level (9.8 ± 3.3 *vs.* 8.6 ± 2.9 × 10^3/mL, respectively; $P = 0.01$), neutrophil count (6.00 ± 2.29 *vs.* 4.43 ± 2.04 × 10^3/mL, respectively; $P < 0.01$) and NLR (3.01 ± 2.34 *vs.* 2.23 ± 1.27, respectively; $P = 0.02$) were significantly higher in CO poisoning group.
Conclusions: The increase of NLR may indicate the progression of fatal complications due to CO poisoning.

1. Introduction

Carbon monoxide (CO) is a toxic gas that consists of one carbon and one oxygen atoms linked by two covalent bonds and one dative covalent bond, with no unpaired electrons[1]. CO poisonings are frequent and can lead to high morbidity and mortality, involving multiple organ systems and undetected CO exposure can be fatal[2]. Neurologic and cardiovascular complications are common. Unfortunately, symptoms are often non-specific and are frequently overlooked[3].

The neutrophil–lymphocyte ratio (NLR) is easy, cheap, noninvasive, and widely available laboratory marker of systemic inflammation. Recently, it gained increased interest due to its role as an independent prognostic factor for many conditions such as uncontrolled hypertension, diabetes mellitus, acute coronary syndromes, valvular heart disease, congenital heart disease, renal or hepatic dysfunction, malignancy, local or systemic infection, and some other inflammatory diseases[4,5]. Whereas high neutrophil counts reflect inflammation, low lymphocyte counts reflect poor general health and physiologic stress[6]. NLR combines these two independent markers of inflammation[7]. In several studies, it has been shown that NLR is an indicator of systemic inflammation[8].

The important role of leukocytes in the pathophysiology of fatal complications due to CO poisoning has been shown in previous studies[9,10]. Moreover, Thom *et al.* report that leukocyte sequestration increases significantly in brain microvasculature following exposure to CO[10]. We speculated that systemic inflammation might play a role in etiopathogenesis of acute CO poisoning. To the best of our knowledge, there is no study investigating the NLR in patients with acute CO poisoning. Therefore, we aimed to investigate NLR as an inflammation marker in patients with CO poisoning.

*Corresponding author: Kenan Ahmet Turkdogan, Adnan Menderes University, Department of Emergency Medicine, 09100, Aydin, Turkey.
Tel: +90 5324833881
E-mail: drturkdogan@gmail.com
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2. Materials and methods

2.1. Patient and methods

There were 569 patients who presented with a diagnosis of CO poisoning to the Emergency Department of Cumhuriyet University School of Medicine, Adnan Menderes University School of Medicine and Isparta State Hospital between June 2009 and March 2014. However, 41 patients were excluded from the study because of exclusion criteria and laboratory mistakes. Finally, the remaining 528 patients (253 male, 275 female; mean age 34 ± 20 years) were included in the study. An age, sex-matched control group was composed of 54 patients (24 women, 30 men with a mean age 39 ± 12 years). The study was approved by the institutional ethics committee and all patients gave their informed consent.

Exclusion criteria were history of acute or chronic renal and liver disease, atherosclerotic heart diseases, heart failure, valvular heart disease, peripheral arterial disease, obesity, diabetes mellitus, history of malignancy, chronic hematological diseases, acute or chronic inflammatory disease, autoimmune disease, drug use affecting NLR. Additionally, patients who were referred to the emergency department because of the need of urgent hyperbaric oxygen treatment and diagnosed died in the emergency department excluded from the study.

Cases were selected from Cumhuriyet University, Adnan Menderes University and Isparta State Hospital between June 2009 and March 2014 database including all patients admitted with diagnosis of CO poisoning. Patients' demographics and medical history including age, gender were abstracted from medical records. Blood samples were withdrawn to determine routine biochemical markers and blood cell analyses. Generally, the blood of patients admitted to the emergency department with the diagnosis of CO poisoning are studied within 15 min. Mean platelet volume (MPV) and other blood samples for platelet indices measurement collected in dipotassium ethylenediaminetetraacetic acid (EDTA) tubes and analyzed with a same automatic blood counter (Beckman Coulter).

2.2. Statistical analysis

Data were analyzed with the SPSS software version 15.0 for Windows. Continuous variables from the study groups were reported as mean ± SD. To compare continuous variables, the student's *t*-test or Mann–Whitney U test were used wherein appropriate. Categorical variables were compared with the X^2 test. Pearson's correlation coefficients were calculated to evaluate relation-ships between variables. A *P* value less than 0.05 was considered as statistically significant.

3. Results

The study group consisted of 528 patients with CO poisoning from stove (275 women, 253 men, mean age 34 ± 20 years). There were 54 patients (24 women, 30 men, mean age 39 ± 12 years) in control group. Patients most commonly admitted between the hours of 8:00 a.m. and 9:00 a.m. (52 patients). The most common admission to the emergency room with the diagnosis of CO poisoning was at December (176 patients, 30.2%).

There was no statistically significant differences between two groups with respect to age ($P = 0.14$) and gender ($P = 0.31$) (Table 1). Aspartate transaminase, potassium, calcium and lymphocytes levels (2.76 ± 1.60 *vs.* 2.43 ± 1.21, respectively; $P = 0.15$) were comparable between CO poisoning group and control group (Table 1). Creatinine ($P = 0.07$) and sodium ($P = 0.06$) levels were slightly significant in both groups. Alanine transaminase level was higher in control group (22 ± 16 *vs.* 27 ± 23, respectively; $P = 0.04$). Alkaline phosphatase (96 ± 59 *vs.* 42 ± 8 IU/L, respectively; $P < 0.01$) and lactate dehydrogenase (159 ± 76 *vs.* 76 ± 14 IU/L, respectively; $P < 0.01$) were significantly higher in CO poisoning group compared with control group (Table 1). Similarly, hemoglobin level (13.7 ± 1.7 *vs.* 12.9 ± 1.1 g/dL, respectively; $P < 0.01$) and platelet counts (275 ± 80 *vs.* 253 ± 64 × 10^9, respectively; $P = 0.04$) were higher in CO poisoning group (Table 2). Moreover, MPV level was significantly higher in CO group (8.6 ± 1.4 *vs.* 8.0 ± 0.7, respectively; $P < 0.01$).

Table 1
Demographic, biochemical characteristics of CO poisoning and control groups.

Characteristics	CO poisoning group (n = 528)	Control group (n = 54)	P
Mean age (year)	34 ± 20	39 ± 12	0.14
Male/Female	253/275	30/24	0.31
Creatinine (mg/dL)	0.92 ± 0.22	0.98 ± 0.30	0.07
Sodium (mg/L)	137 ± 3	138 ± 3	0.06
K (mg/L)	4.0 ± 0.5	4.1 ± 0.4	0.45
AST (U/L)	27 ± 25	27 ± 15	0.87
ALT (U/L)	22 ± 16	27 ± 23	0.04
Ca (mg/L)	9.2 ± 0.5	9.4 ± 0.6	0.17
ALP (IU/L)	96 ± 59	42 ± 8	< 0.01
LDH (IU/L)	159 ± 76	76 ± 14	< 0.01

Table 2
Hematologic characteristics of CO poisoning and control groups.

Characteristics	CO group (n = 528)	Control group (n = 54)	P
Hemoglobin (g/dL)	13.70 ± 1.70	12.90 ± 1.10	< 0.01
WBC × 10^3/mL	9.80 ± 3.30	8.60 ± 2.90	0.01
Platelet × 10^3/mm^3	275.00 ± 80.00	253.00 ± 64.00	0.04
MPV (fL)	8.60 ± 1.40	8.00 ± 0.70	< 0.01
Neutrophils × 10^3/mL	6.00 ± 2.29	4.43 ± 2.04	< 0.01
Lymphocytes × 10^3/mL	2.76 ± 1.60	2.43 ± 1.21	0.15
NLR	3.01 ± 2.34	2.23 ± 1.27	0.02

The markers associated with inflammations; white blood cell (WBC) level (9.8 ± 3.3 *vs.* 8.6 ± 2.9 × 10^3/mL, respectively; $P = 0.01$) and neutrophil count (6.00 ± 2.29 *vs.* 4.43 ± 2.04 × 10^3/mL, respectively; $P < 0.01$) were significantly higher in CO poisoning group than control group. Moreover, NLR was also significantly higher in CO poisoning group (3.01 ± 2.34 *vs.* 2.23 ± 1.27, respectively; $P = 0.02$) (Table 2).

In correlation analysis, the NLR was positively correlated with neutrophil count ($P < 0.01$, $r = 0.69$) and WBC level ($P < 0.01$, $r = 0.35$). Unlikely, it was negatively correlated with lymphocyte count ($P < 0.01$, $r = 0.56$).

4. Discussion

In the present study, we examined indices associated with inflammation in patients with acute CO poisoning. We found that WBC, MPV, neutrophil count and NLR were significantly

higher in patients with CO poisoning. More importantly, NLR was positively correlated with neutrophil count, WBC level.

The NLR is easy, cheap, noninvasive, and widely available laboratory marker of systemic inflammation. Recently, it gained increased interest due to its role as an independent prognostic factor for many conditions such as uncontrolled hypertension, diabetes mellitus, acute coronary syndromes, valvular heart disease, congenital heart disease, renal or hepatic dysfunction, malignancy, local or systemic infection, and some other inflammatory diseases[4,5]. The NLR is a combination of 2 independent markers of inflammation: neutrophils, as a marker of ongoing nonspecific inflammation, and lymphocytes, as a marker of the regulatory pathway[11]. The combination of these 2 markers, the NLR, has proved to a powerful simple marker of inflammation[12].

Several mechanisms have been postulated in the pathophysiological mechanisms of CO poisoning. As regards the specific mechanisms, CO is capable to bind to the heme group of myoglobin with an affinity of 60-times greater than that of oxygen, thus reducing the oxygen supply to the mitochondria, impairing the oxidative phosphorylation and deteriorating the energy source of myocardium[13]. CO is also directly toxic for mitochondria, through impairment of mitochondrial respiratory chain at the cytochrome *c* oxidase level[14]. This binding of CO to the hemoglobin molecule causes alterations in the hemoglobin molecule, preventing oxygen from being released easily, which causes a reduction in oxygen delivery to the tissues, resulting in tissue hypoxia[15]. Thus, neurological and cardiovascular manifestations are observed[16]. To the best of our knowledge, there is no study directly investigating the inflammation marker on acute complications due to CO poisoning. The pathophysiologic mechanisms underlying these complications are still not fully understood. One of these mechanisms is that CO poisoning activates nitric oxide and other oxygen free radicals[17]. Oxygen free radicals can affect blood flow, contributing to endothelial damage[18]. It is postulated that this oxidative injury is mediated largely by leukocytes. Moreover, in rats made leukopenic, lipid peroxidation is inhibited following CO poisoning. Leukocyte sequestration increases significantly in brain microvasculature following exposure to CO[10]. The generation of oxygen radicals during reperfusion has been implicated as the major component of post-ischemic brain injury[9]. In previous studies, it has shown that neutrophils play a role in CO-mediated brain injuries in CO poisoning[10,19]. In present study, the number of circulating neutrophil was significantly higher in CO poisoning group. However, lymphocyte count was comparable in both groups.

A second mechanism, increased thrombotic tendency has been reported in patients with CO poisoning[20,21]. Thom *et al.* shown that acute CO poisoning causes intravascular neutrophil activation due to interactions with platelets[22]. Similarly, we found that platelet and MPV level were significantly higher in CO poisoning patients. We anticipate that the results of our study will indirectly support that study. Nevertheless, we did not know the exact cause of increased NLR in patients with CO poisoning. Further prospective studies are needed to establish the pathophysiological and clinical significance of increased NLR in patients with CO poisoning.

There are several limitations in this study. Firstly, this study was conducted on a retrospective basis and represented only three-center experience. Secondly, our analysis was based on a simple baseline determination at a single time point that may not reflect patient status over long periods. Thirdly, evaluation of MPV within 30 min is recommended when blood sample is collected in EDTA tube. In our study, although it is retrospective, blood samples of patients admitted with the diagnosis of CO poisoning to the emergency department are usually studied within 15 min. Finally, our study population may be small. Accordingly, it may limit the statistical power of the study.

In conclusion, we found that the NLR significantly elevated in patients with CO poisoning. We suggested that systemic inflammation may be effective in the development of complications due to CO poisoning. Moreover, the increase of NLR may indicate the progression of complications due to CO poisoning. Therefore, anti-inflammatory drug usage in acute CO poisoning might be reasonable.

Conflict of interest statement

The authors report no conflict of interest.

References

[1] Turkdogan KA, Karabacak M, Kapci M, Akpinar O. Rare cause of acute myocardial infarction; carbon monoxide poisoning. *Acta Medica Mediterr* 2014; **30**: 743-5.
[2] Alharbi Z, Grieb G, Pallua N. Carbon monoxide intoxication in burns. In: McLaughlin ES, Paterson AO, editors. *Burns: prevention, causes and treatment*. New York: Nova Science Publishers; 2012, p. 171-80.
[3] Weaver LK. Clinical practice. Carbon monoxide poisoning. *N Engl J Med* 2009; **360**(12): 1217-25.
[4] Balta S, Demirkol S, Cakar M, Arslan Z, Unlu M, Celik T. Other inflammatory markers should not be forgotten when assessing the neutrophil-to-lymphocyte ratio. *Clin Appl Thromb Hemost* 2013; **19**(6): 693-4.
[5] Sahin DY, Elbasan Z, Gur M, Yildiz A, Akpinar O, Icen YK, et al. Neutrophil to lymphocyte ratio is associated with the severity of coronary artery disease in patients with ST-segment elevation myocardial infarction. *Angiology* 2013; **64**(6): 423-9.
[6] Gibson PH, Cuthbertson BH, Croal BL, Rae D, El-Shafei H, Gibson G, et al. Usefulness of neutrophil/lymphocyte ratio as predictor of new-onset atrial fibrillation after coronary artery bypass grafting. *Am J Cardiol* 2010; **105**(2): 186-91.
[7] Shen XH, Chen Q, Shi Y, Li HW. Association of neutrophil/lymphocyte ratio with long-term mortality after ST elevation myocardial infarction treated with primary percutaneous coronary intervention. *Chin Med J (Engl)* 2010; **123**(23): 3438-43.
[8] Kaya MG, Akpek M, Elcik D, Kalay N, Yarlioglues M, Koc F, et al. Relation of left atrial spontaneous echocardiographic contrast in patients with mitral stenosis to inflammatory markers. *Am J Cardiol* 2012; **109**(6): 851-5.
[9] Thom SR, Bhopale VM, Han ST, Clark JM, Hardy KR. Intravascular neutrophil activation due to carbon monoxide poisoning. *Am J Respir Crit Care Med* 2006; **174**(11): 1239-48.
[10] Thom SR. Leukocytes in carbon monoxide-mediated brain oxidative injury. *Toxicol Appl Pharmacol* 1993; **123**(2): 234-47.
[11] Núñez J, Núñez E, Bodí V, Sanchis J, Miñana G, Mainar L, et al. Usefulness of the neutrophil to lymphocyte ratio in predicting long-term mortality in ST segment elevation myocardial infarction. *Am J Cardiol* 2008; **101**(6): 747-52.
[12] Horne BD, Anderson JL, John JM, Weaver A, Bair TL, Jensen KR, et al. Which white blood cell subtypes predict increased cardiovascular risk? *J Am Coll Cardiol* 2005; **45**(10): 1638-43.
[13] Prockop LD, Chichkova RI. Carbon monoxide intoxication: an updated review. *J Neurol Sci* 2007; **262**(1–2): 122-30.
[14] Alonso JR, Cardellach F, López S, Casademont J, Miró O. Carbon monoxide specifically inhibits cytochrome c oxidase of human

mitochondrial respiratory chain. *Pharmacol Toxicol* 2003; **93**(3): 142-6.

[15] Ernst A, Zibrak JD. Carbon monoxide poisoning. *N Engl J Med* 1998; **339**(22): 1603-8.

[16] Olson KR. Carbon monoxide poisoning: mechanisms, presentation, and controversies in management. *J Emerg Med* 1984; **1**(3): 233-43.

[17] Ruth-Sahd LA, Zulkosky K, Fetter ME. Carbon monoxide poisoning: case studies and review. *Dimens Crit Care Nurs* 2011; **30**(6): 303-14.

[18] Felner JM, Churchwell AL, Murphy DA. Right atrial thromboemboli: clinical, echocardiographic and pathophysiologic manifestations. *J Am Coll Cardiol* 1984; **4**(5): 1041-51.

[19] Ischiropoulos H, Beers MF, Ohnishi ST, Fisher D, Garner SE, Thom SR. Nitric oxide production and perivascular tyrosine nitration in brain following carbon monoxide poisoning in the rat. *J Clin Invest* 1996; **97**(10): 2260-7.

[20] Marius-Nunez AL. Myocardial infarction with normal coronary arteries after acute exposure to carbon monoxide. *Chest* 1990; **97**(2): 491-4.

[21] Karabacak M, Varol E, Turkdogan KA, Duman A, Akpinar O, Karabacak P. Mean platelet volume in patients with carbon monoxide poisoning. *Angiology* 2014; **65**(3): 252-6.

[22] Thom SR, Fisher D, Manevich Y. Roles for platelet-activating factor and *NO-derivedoxidants causing neutrophil adherence after CO poisoning. *Am J Physiol Heart Circ Physiol* 2001; **281**(2): H923-30.