Serum Netrin-1 and Asymmetric Dimethylarginine as Biomarkers of Acute and Chronic Carbon Monoxide Poisoning

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KEYWORDS
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Asymmetric dimethylarginine (ADMA),
Carbon monoxide,
Poisoning,
Biomarkers,
Clinical study.

ABSTRACT

Diagnosis of carbon monoxide (CO) poisoning is done traditionally by detecting carboxyhemoglobin (COHb) level. However, the severity of clinical manifestations is unrelated to the absolute levels of COHb. Netrin-1 (Net-1) is a protein with anti-inflammatory and anti-apoptotic properties while asymmetric dimethylarginine (ADMA) is an oxidative stress indicator. This study aimed at assessing and comparing between Net-1, ADMA and COHb as biomarkers in CO poisoning. This cross-sectional clinical study was carried out on 30 acutely CO poisoned adults admitted to Benha Poisoning Control Unit, Benha University Hospitals; 30 chronic CO exposed workers at four different car service centres, plus 30 healthy controls. Results showed a positive correlation between serum levels of Net-1 and ADMA and the severity of acute CO poisoning both on admission, and after oxygen therapy. There was a positive correlation between COHb level and both serum levels of Net-1 and ADMA in chronic cases. In conclusion: COHb level can only support the initial diagnosis of acute CO poisoning. Both ADMA and Net-1 are better biomarkers in assessing the severity of acute CO poisoning, where ADMA is better than Net-1. In diagnosis of chronic CO poisoning, both ADMA and Net-1 are as dependable as COHb.

Introduction

Carbon monoxide (CO) is an odorless, colorless, nonirritant gas, it is lighter than air, and produced by partial combustion of carbon containing compounds. It is often called the "silent killer" as victims of CO poisoning lose their consciousness even before they realize that they are being poisoned (Jung and Lee, 2019).

Both acute and chronic poisonings occur due to CO exposure; acute poisoning results from inhalation of CO-containing air in closed spaces, whereas chronic poisoning is detected among people working in industries as a result of continuous prolonged exposure to low CO concentrations (Koyuncu et al., 2019).

Acute CO poisoning affects multiple organ systems with no typical clinical picture (non-specific presentation); therefore, misdiagnosis is a common possibility (Reumuth et al., 2019).

Chronic CO poisoning has rarely been reported despite being more prevalent and

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associated with increased morbidity and mortality than previously reported (Bol et al., 2018).

Pathological effects of CO poisoning are caused by hypoxia, in addition, CO poisoning produces reactive oxygen species, and oxidative stress that induces immunological and inflammatory damages to all body organs (Huang et al., 2019).

Diagnosis of CO poisoning is done by detecting the percentage of carboxyhemoglobin (COHb) in the arterial blood. However, the severity of clinical picture is unrelated to the absolute levels of COHb, which is the routine biomarker of CO poisoning (Reumuth et al., 2019).

Therefore, detection of new and better biomarkers that can diagnose the severity and outcome of CO poisoning is essential (Yazar et al., 2019).

Netrin-1 (Net-1) is a laminin-related protein that alleviates cellular infiltration and inflammation by suppressing inflammatory cytokines and chemokines. Net-1 inhibits the apoptosis of endothelial and neuronal cells and improves neuronal and vascular regeneration after ischemic injury (Gedikli et al., 2019; Guo et al., 2019).

Asymmetric dimethylarginine (ADMA) has structural similarity to the amino acid L-arginine. It is an oxidative stress indicator as it inhibits the synthase of nitric oxide. So, levels of ADMA increase in the course of oxidative stress, inflammation, and endothelial dysfunction (Liyona et al., 2019).

Few published researches studied the role of either Net-1 or ADMA in diagnosis of acute CO poisoning; and no study was conducted to compare between Net-1 and ADMA as diagnostic biomarkers of both acute and chronic CO poisonings.

Therefore this work aimed at assessing the role of serum Net-1 and ADMA levels as possible biomarkers for diagnosis of both acute and chronic CO poisoning; determining the correlation between serum Net-1 and ADMA levels and the severity of acute CO poisoning, and comparing between Net-1, ADMA and COHb levels as biomarkers in CO poisoning.

Subjects and Methods

This cross-sectional clinical study was carried out on 60 cases of both acute and chronic CO poisoning, plus 30 healthy controls, as follows:

Thirty adult patients (aged >18 years) admitted to Benha Poisoning Control Unit (BPCU), Benha University Hospitals, Egypt, with acute CO poisoning. Thirty chronic CO exposed male workers; working at four different car service centres for at least five years, six work shifts/week, 8 hours/shift, of age >18 years, in Benha City, Qalubia Governorate, Egypt. Thirty healthy volunteers of the same age and sex distribution as test group, were enrolled in the study as a control group to determine the mean average normal values of COHb, Net-1 and ADMA levels.

Exclusion criteria:

1. Patients who are proven by history, physical examination, or investigations to have any chronic inflammatory conditions or chronic diseases such as cardiovascular, lung, kidney, liver diseases, haemolytic anaemia and malignancy.

2. Smoking.
3. Patients refused to give informed consent, or their relatives do so [in unconscious patients].
4. Pregnancy.

All included subjects were evaluated according to the followings: -

1- Demographic study: age, gender, home heating devices, plus duration of daily exposure (in chronic cases).

2- Clinical study:

- Clinical manifestations detected by history taking and physical examination.
- Severity of poisoning in acute patients either at admission (0^{th} h) or after oxygen therapy (6^{th} h), was classified according to poisoning severity score (PSS), describe by Persson et al. (1998).
- Treatment measures done to acute patients; according to Tomaszewski (2006). All patients were treated using normobaric oxygen as hyperbaric oxygen was not available.

3-Investigational study:

A) Blood samples: in acute cases, blood samples were taken twice; the first samples were taken at admission of patient (0^{th} h), the second samples were taken after oxygen therapy [6^{th} h], meanwhile in chronic cases, blood samples were taken once, at mid-day work, from workers presenting with any of the chronic manifestations known by history taking.

B) Carboxyhemoglobin (COHb) levels:

- Immediately after collection of 2 ml of arterial blood samples; COHb level was measured by a spectrometry assay and expressed as a percentage of plasma hemoglobin, using a blood gas analyzer [ABL800 FLEX blood gas analyser (Radiometer America Inc.)] with a multiwavelength spectrometer, absorbing the visible light from 478 to 672 nm as described by Bono et al. (2007) and Crapo et al. (1999).
- COHb level >5% was considered as diagnostic criteria for CO poisoning, as described by Koyuncu et al. (2019).

C) Serum netrin-1 (Net-1) and serum asymmetric dimethyl arginine (ADMA) levels:

- Three ml venous blood samples were collected in Vacutainer tubes and allowed to clot.
- Both were measured by Enzyme-Linked Immunosorbent Assay (ELISA) technique, based on the Biotin double antibody sandwich technology, by BioRad PR4100® (France) ELISA plate reader device.
- Serum Net-1 levels were measured using human Netrin-1 ELISA Kit, Bioassay (China), Cat# E1277Hu, following the manufacturer's protocol, as described by Ramesh et al. (2011). Standard detection curve range: 10 pg/ml – 3000 pg/ml.
- Serum ADMA levels were measured using human asymmetrical dimethylarginine (ADMA) ELISA Kit, Bioassay (Shanghai Crystal Day Biotech Co., Ltd. China), Cat# E4539Hu, following the manufacturer's instructions, as described by Schulze et al. (2004). Assay range: 0.05nmol/ml-4nmol/ml.

The study protocol was approved by the Research Ethics Committee of Benha Faculty of Medicine, Benha University

[certificate No. 1017; 2019]. An informed consent was taken from each participant in this study. All results obtained were registered in special sheets of the study, which were confidential.

Statistical analysis

The collected data were analyzed using SPSS version 21 software (SpssInc, Chicago, ILL Company). Categorical data were presented as number and percentages. Quantitative data were expressed as mean ± standard deviation if normally distributed or median and range if not. Man Whitney U test (Z_{MWU}) was used for nonparametric variables among two independent groups, while Kruskal Wallis test (KW) for three independent groups. Matched nonparametric variables were assessed by Wilcoxon test. Nonparametric correlations were assessed by Spearman's correlation coefficient (rho). ROC curves were constructed to determine cutoff values of the studied biomarkers with optimum sensitivity and specificity in prediction of severity grades. P ≤0.05 was considered significant (Khothari, 2004).

Results

Majority of acute CO poisoning cases (83.3%) were males. The mean age of cases was 34.8 ±10.7 (ranging from 19 to 51 years). Heating devices were found at homes of about 81% of cases.

All chronic CO exposure cases were males, with a mean age of 38.6 ±10.3 (ranging from 24 to 55 years). Heating devices were found at homes of 47% of cases. The mean duration of daily CO exposure was 5.2 ±0.86, with a range of 4-6 hours.

The most common representing manifestations of acute CO poisoning cases were headache (96.7%), dizziness (90.0%) and confusion (66.7%). On admission, and according to PSS, 15 patients (50%) were mild, ten patients (33.3%) were moderate and five patients (16.7%) were severe. Fourteen patients (46.7%) were treated with 100% O2 applied by non-rebreather (reservoir) face mask; 11 patients (36.7%) were treated with O2 by nasal canula or nasal mask and five patients (16.7%) were treated with 100% O2 applied by endotracheal tube. Most of patients (53.3%) became asymptomatic after O2 therapy and were discharged.

Most of cases with chronic CO exposure were manifested by persistent headache (66.7%); palpitation (60%); dyspnea (56.7%) and dizziness (43.3%).

As regard cases of acute CO poisoning: the median serum level of COHb on admission (0th h) was 9.5%, which was significantly high ($p < 0.001$) as compared with controls (2.0%), meanwhile the median serum level of COHb after oxygen therapy (6^{th} h) was 2.0%, which was significantly decreased ($p < 0.001$), as compared with 0^{th} h level, (details showed in Table 1).

Table (1): Comparison between cases of acute CO poisoning and controls regarding serum levels of carboxyhemoglobin (COHb); netrin-1 (Net-1) and asymmetric dimethyl arginine (ADMA) on admission (0^{th} h) and after oxygen therapy (6^{th} h):

Variable	Controls (n=30)		0^{th} h (n=30)		6^{th} h. (n=30)		Z_{MWU1} (P)	Wilcoxon test (P)	Z_{MWU2} (P)
	Median	Range	Median	Range	Median	Range			
COHb	2.0	0-5	9.5	5-22	2.0	1-5	6.67 ***(<0.001)	4.79 ***(<0.001)	0.98 (0.32)
Net-1 (pg/ml)	400.5	337-474	715.0	600-1200	779.5	634-1166	6.65 ***(<0.001)	4.64 ***(<0.001)	6.65 ***(<0.001)
ADMA (nmol/L)	0.71	0.6-0.95	0.855	0.6-1.97	1.58	0.98-3.16	3.98 ***(<0.001)	4.78 ***(<0.001)	6.65 ***(<0.001)

Z_{MWU1} (P): between controls and acute 0 h**; Wilcoxon test (P):** between acute at 0 and 6 h; **Z_{MWU2} (P)**: between controls and acute 6 h; ***** :** highly significant; **COHb :** carboxyhemoglobin; **ADMA:** asymmetric dimethyl arginine; **Net-1:** netrin-1

Meanwhile, on admission, the median serum levels of Net-1 and ADMA were 715 pg/mL and 0.855 nmol/L, respectively, which were significantly high ($p < 0.001$) as compared with controls (400.5 pg/mL and 0.71 nmol/L, respectively), meanwhile the median serum levels of Net-1 and ADMA after oxygen therapy were 779.5 pg/mL and 1.58 nmol/L, respectively, which were significantly increased ($p < 0.001$) as compared with 0^{th} h levels, (details showed in table 1).

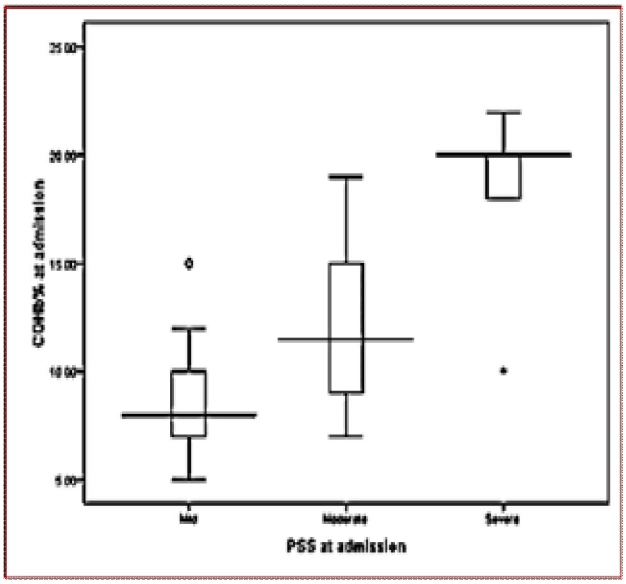
There was a positive correlation between serum levels of COHb and severity of acute CO poisoning, which was statistically significant ($p < 0.001$) at 0^{th} h, meanwhile it became non-significant at 6^{th} h, (details showed in table 2 and figure 1).

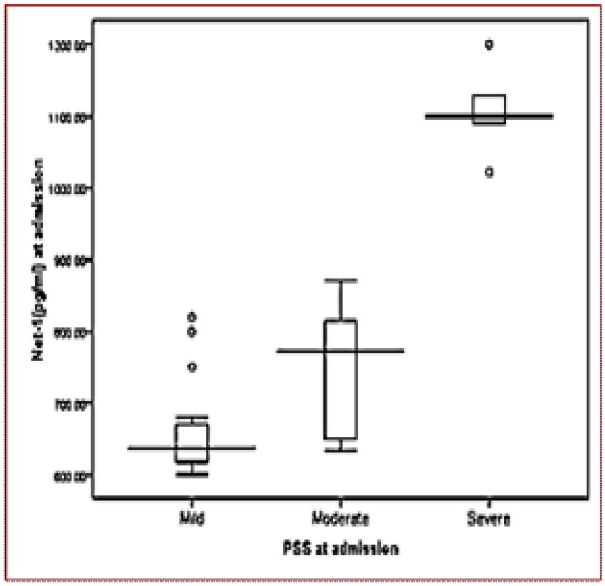
Table (2): Spearman's correlation coefficient between the studied biomarkers (COHb; Net-1 and ADMA) and the poisoning severity score (PSS), at admission (0^{th} h) and after oxygen therapy (6^{th} h) in acute CO poisoning cases:

PSS at admission (0^{th} h) (n=30)		
Variable	**rho**	**p value**
COHb	0.650	***<0.001 (HS)
Net-1 (pg/ml)	0.731	***<0.001 (HS)
ADMA (nmol/L)	0.810	***<0.001 (HS)
PSS at admission (6^{th} h) (n=30)		
Variable	**rho**	**p value**
COHb	0.089	0.64 (NS)
Net-1 (pg/ml)	0.736	***<0.001 (HS)
ADMA (nmol/L)	0.787	***<0.001 (HS)

*****:** Very highly significant; **COHb:** carboxyhemoglobin; **ADMA:** asymmetric dimethyl arginine; **Net-1:** netrin-1; **PSS:** poisoning severity score; **rho:** spearman's correlation coefficient; **HS:** highly significant; **NS:** non-significant.

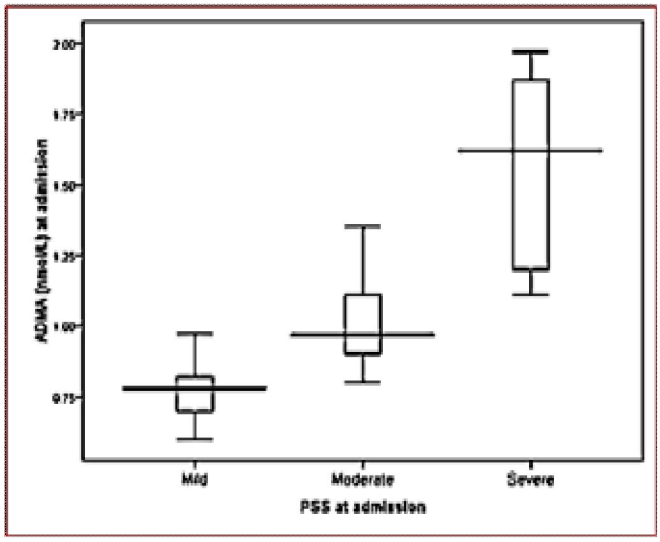
There was a positive correlation between serum levels of Net-1 and ADMA and the severity of acute CO poisoning, this correlation was found to be statistically significant ($p < 0.001$) at 0^{th} h, and at 6^{th} h, (details showed in table 2 and figure 2).





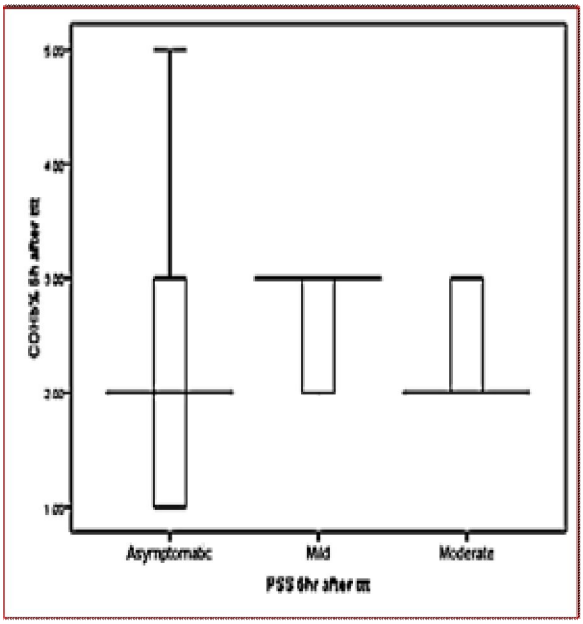
Median:	8.0	11.5	20.0
Range:	5-15	7-19	10-22

Median:	637.0	773.0	1100.0
Range:	600-820	634-871	1022-1200

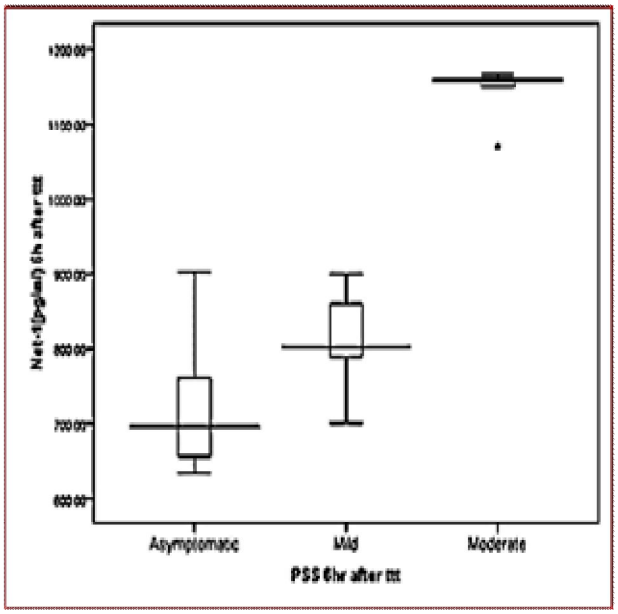


Median:	0.78	0.97	1.62
Range:	0.6-0.97	0.8-1.35	1.11-1.97

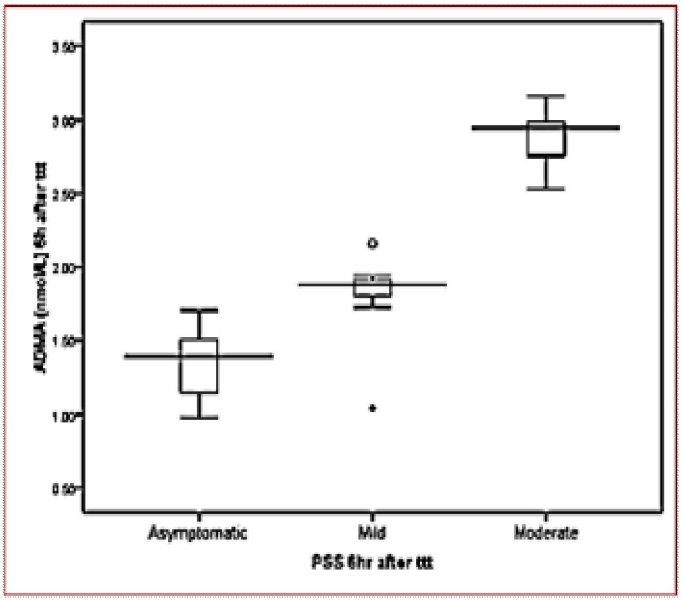
Fig. (1): Box plot showing the median and range of studied biomarkers [carboxyhemoglobin (COHb); netrin-1 (Net-1) and asymmetric dimethylarginine (ADMA)] in relation to the poisoning severity score (PSS) of acute CO poisoning cases on admission (0^{th} h).



Median: 2.0 3.0 2.0
Range: 1-5 2-3 2-3



Median: 695.5 802.0 1159
Range: 634-902 700-900 1070-116



Median: 1.39 1.88 2.95
Range: 0.98-1.7 1.04-2.16 2.53-3.16

Fig. (2): Box plot showing the median and range of studied biomarkers [carboxyhemoglobin (COHb); netrin-1 (Net-1) and asymmetric dimethylarginine (ADMA)] in relation to the poisoning severity score (PSS), after oxygen therapy (6^{th} h) of acute CO poisoning cases.

Receiver operator characteristic (ROC) curves were done to compare between serum Net-1, ADMA and COHb levels as biomarkers in acute CO poisoning, results illustrated that Net-1 and ADMA were more accurate and sensitive biomarkers as compared with COHb. The ROC curves illustrated also that ADMA is more accurate and sensitive biomarker as compared with Net-1. These results were statistically significant (p <0.001), (details showed in tables 3 & 4 and figures 3 & 4).

Table (3): Cutoff values, area under the curve (AUC) and confidence interval (CI) of receiver operator characteristic (ROC) curve for comparing accuracy and performance of the studied biomarkers to differentiate moderate/severe from mild cases of acute CO poisoning on admission (0^{th} h):

Score	Cutoff values	Sensitivity %	Specificity %	PPV %	NPV %	AUC	95% CI	p value
COHb	≥9.5	73.3%	73.3%	73.3%	73.3%	0.833	0.68-0.98	**0.002
Net-1(pg/ml)	≥715	80%	80%	80%	80%	0.867	0.74-0.99	**0.001
ADMA (nmol/L)	≥0.88	86.7%	93.3%	92.8%	87.5%	0.933	0.85-1.0	***<0.001

PPV: positive predictive value; **NPV:** negative predictive value; ** **:** highly significant; ***: very highly significant; **AUC:** area under the curve; **CI:** confidence interval; **COHb:** carboxyhemoglobin; **ADMA:** asymmetric dimethyl arginine; **Net-1:** netrin-1.

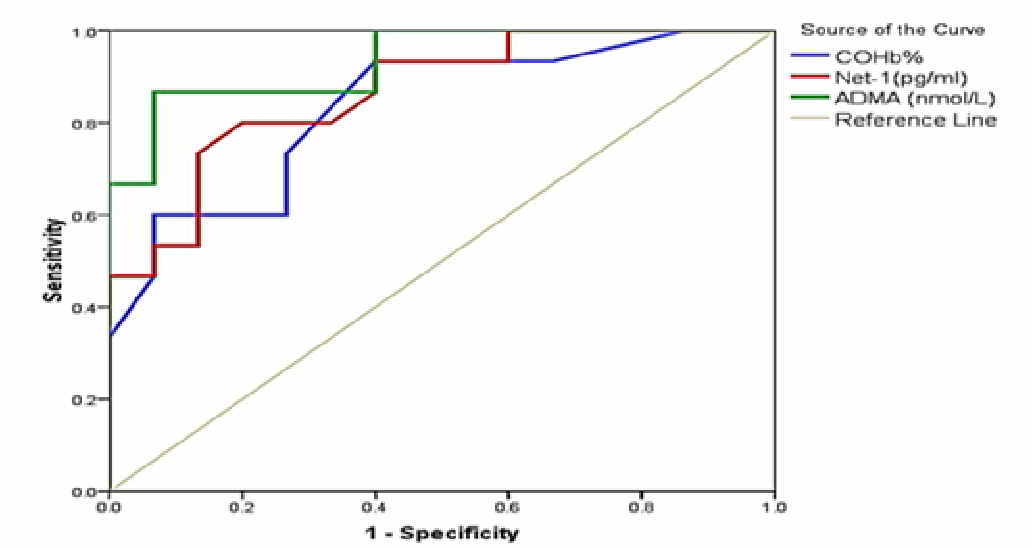


Fig. (3): Receiver operator characteristic (ROC) curve for comparing accuracy and performance of the studied biomarkers [carboxyhemoglobin (COHb); netrin-1 (Net-1) and asymmetric dimethylarginine (ADMA)] to differentiate moderate/severe from mild cases of acute CO poisoning on admission (0^{th} h). Related cutoff values, area under the curve (AUC) and confidence interval (CI) of receiver operator characteristic (ROC) were illustrated in table (3).

Table (4): Cutoff values, Area Under the Curve (AUC) and Confidence Interval (CI) of receiver operator characteristic (ROC) curve for comparing accuracy and performance of the studied biomarkers to differentiate moderate/severe from mild cases of acute CO poisoning after oxygen therapy (6^{th} h):

Score	Cutoff values	Sensitivity %	Specificity %	PPV %	NPV %	AUC	95% CI	p value
COHb	≥2.5	50%	56.2%	50%	56.2%	0.562	0.35-0.78	0.56
Net-1(pg/ml)	≥779.5	85.7%	81.2%	80%	86.7%	0.877	0.75-1.0	***<0.001
ADMA (nmol/L)	≥1.67	92.9%	93.8%	92.9%	93.8%	0.902	0.77-1.0	***<0.001

PPV: positive predictive value; **NPV:** negative predictive value; ***: very highly significant; **AUC:** area under the curve; **CI:** confidence interval; **COHb:** carboxyhemoglobin; **ADMA:** asymmetric dimethyl arginine; **Net-1:** netrin-1.

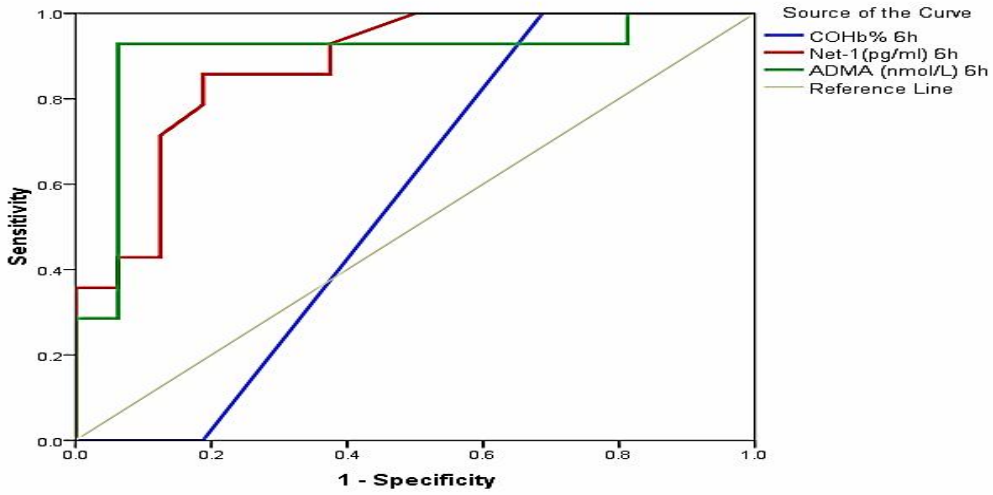


Fig. (4): ROC curve for comparing accuracy and performance of the studied biomarkers [carboxyhemoglobin (COHb); netrin-1 (Net-1) and asymmetric dimethylarginine (ADMA)] to differentiate mild and moderate acute CO poisoning cases from asymptomatic cases after oxygen therapy (6^{th} h). Related cutoff values, area under the curve (AUC) and confidence interval (CI) of receiver operator characteristic (ROC) were illustrated in table (4).

Table (5): Comparison between serum levels of carboxyhemoglobin (COHb); netrin-1 (Net-1) and asymmetric dimethyl arginine (ADMA) of chronic CO exposed cases and controls:

Variables	Controls (n=30)		Chronic (n=30)		Z_{MWU} (P)
	Median	Range	Median	Range	
COHb	2.0	0-5	8.0	5-11	6.68 ***(<0.001)
Net-1 (pg/ml)	400.5	337-474	749.0	613-975	6.65 ***(<0.001)
ADMA (nmol/L)	0.71	0.6-0.95	1.27	0.75-2.0	6.24 ***(<0.001)

***: very highly significant; **COHb:** carboxyhemoglobin; **ADMA:** asymmetric dimethyl arginine; **Net-1:** netrin-1.

There was a positive correlation between the COHb level and both serum levels of Net-1 and ADMA in chronic cases; this correlation was found to be statistically significant ($p < 0.001$), details are showed in table (6).

Table (6): Spearman's correlation coefficient between the studied biomarkers (COHb; Net-1 and ADMA) in chronic CO exposed cases:

Variables		*COHb*	*Net-1 (pg/ml)*	*ADMA (nmol/L)*
Net-1 (pg/ml)	rho	0.603	----	0.557
	P	***<0.001	----	**=0.001
ADMA (nmol/L)	rho	0.495	0.557	-----
	P	**0.005	**=0.001	-----

***: very highly significant; **: highly significant; **COHb:** carboxyhemoglobin; **ADMA:** asymmetric dimethyl arginine; **Net-1:** netrin-1.

Discussion

Pathogenesis of CO poisoning, either in acute or chronic exposure, occurs mainly by two mechanisms; the first mechanism is tissue hypoxia, especially in organs with the highest oxygen demand like the brain and the heart, due to formation of COHb, CO can also bind myoglobin and mitochondrial cytochrome oxidases leading to more tissue hypoxia. The second mechanism occurs by production of reactive oxygen species and oxidative stress damages, these effects are longer lasting and independent of hypoxia (Huang et al., 2019; Jung and Lee, 2019).

Eichhorn et al. (2018) concluded that long term pathological tissue damages are detected as a result of acute CO poisoning, despite O_2 therapy that lead to drop of COHb levels to normal values. They justified these delayed tissue injuries by the effects of CO poisoning at cellular level, where it leads to the activation of neutrophils, proliferation of lymphocytes, mitochondrial dysfunction, oxidative stress, inflammation, and apoptosis.

Masters et al. (2019) found that clinical diagnosis of CO poisoning is difficult due to its vague symptoms especially in atypical presentations. They also explained the under-diagnosis of CO poisoning to gaps present between the capacity to detect the blood level of COHb and the unavailability of other confirmatory tests.

Veronesi et al. (2017) stated that endogenous production of COHb can occur as a result of some diseases, such as chronic obstructive lung disease, liver cirrhosis or transplantation and haematological diseases such as haemolytic anaemia.

The present study found that on admission (0^{th} h) of cases with acute CO poisoning, the median serum level of COHb was 9.5%, which was significantly high as compared with controls (2.0%), meanwhile it was significantly decreased after O2 therapy (6^{th} h). The present work also found a statistically significant positive correlation between serum levels of COHb and severity of acute CO poisoning, only at 0^{th} h, meanwhile it became non-significant at 6^{th} h after O_2 therapy.

Reumuth et al. (2019) concluded that COHb level alone is an insufficient marker for assessing the severity of acute CO poisoning. They also stated that high COHb level can only support the initial diagnosis of CO poisoning, and further biomarkers are needed to assess the severity of CO poisoning.

Yazar et al. (2019) showed that there was a poor correlation between COHb levels and

the clinical symptoms and final outcome of acute CO poisoning cases. They reach to a conclusion that increased COHb blood levels can help in initial diagnose of acute CO poisoning but do not conclude possible long-term neuropsychiatric or cardiac consequences. Therefore, finding new and better biomarkers that can predict the duration of treatment and outcome of CO poisoning is essential.

The current work showed that, in cases of chronic CO exposure, the median serum level of COHb was 8.0%, which was significantly high, as compared with controls (2.0%). This can be justified as this study was done on workers at four different car service centers (8 h./shift, for at least 5 years); blood COHb estimation was done at mid-day work where CO exposure was at its highest level.

Nair et al. (2017) concluded that CO gas is considered as a toxic hazard for car service workers, as it is one of the pollutants from vehicle exhaust which is usually released due to incomplete combustion of fuel.

World Health Organization stated that, in chronic CO exposure, an acceptable blood level of COHb should not below the level of 2.5% depending on the CO levels in the environment and duration of exposure (Bol et al., 2018).

ADMA is present as a natural metabolite in human plasma, and it is considered as an oxidative stress index, it blocks nitric oxide (a potent vasodilator) biogenesis and limit the cellular uptake of L-arginine, and this results in impairment of the endothelial function, leading to promotion of ischemic atherosclerosis (Dymara-Konopka and Laskowska, 2019).

Yazar et al. (2019); Oliva-Damaso et al. (2019) concluded that ADMA is synthesized from arginine by the action of the enzyme protein arginine methyltransferases, while degradation of ADMA occurs by dimethyl arginine dimethylaminohydrolase enzyme. In the course of oxidative stress, whatever its cause, there will be activation of protein arginine methyltransferases enzyme and inhibition of dimethyl arginine dimethylaminohydrolase, leading to increased level of ADMA.

Moreover, high levels of ADMA result in generation of nitric superoxide and peroxynitrite free radicals. These free radicals will inactivate mitochondrial enzymes and cause cell membrane lipid peroxidation with further damage to vascular endothelium (Abass et al., 2017).

The present study found that on admission (0^{th} h) of acute CO poisoning cases, the median serum level of ADMA was significantly high as compared with controls, meanwhile it was significantly increased after O_2 therapy (6^{th} h) as compared with its level on admission. There was a significant positive correlation between serum levels of ADMA and the severity of acute CO poisoning, at both 0^{th} h, and at 6^{th} h. The present work also showed that in cases of chronic CO exposure, the median serum level of ADMA was significantly increased as compared with controls, with a significant positive correlation between the COHb levels and serum ADMA levels.

The elevated serum levels of ADMA in either cases of acute or chronic CO exposure can be justified as ADMA is an oxidative stress biomarker that is elevated as a part of CO-induced progressive oxidative stress.

Yazar et al. (2019) concluded that ADMA levels were significantly increased in patients with CO poisoning on admission and after O_2 therapy when compared with controls, with a positive relation between serum levels of ADMA and the severity of CO poisoning.

Net-1 acts as an anti-inflammatory molecule through inhibition of leukocyte infiltration and cytokine production especially

in conditions precipitated by hypoxia and/or oxidative stress. Net-1 has neuroprotective functions including preservation of blood–brain barrier integrity, anti-inflammatory and anti-apoptotic effects (Sun et al., 2019).

Developmentally, Net-1 is a chemical regulator of neural axon guidance and angiogenesis, it has a neuroprotective effect in cases of ischemic brain injury (Chen et al., 2019).

Lin et al. (2018) stated that Net-1 has key roles in neuronal navigation, immune cell migration, angiogenesis, and cell survival. In endothelial cells, Net-1 stimulates nitric oxide production and promotes endothelial cell migration and proliferation.

Daliang et al. (2019) concluded that Net-1 at high levels can improve myocardial ischemic-reperfusion injuries, as it can activate nitric oxide synthase enzyme, leading to induction nitric oxide production that in turn protects against ischemic injuries and reduce the area of infarction.

The present study showed that on admission (0^{th} h) of cases with acute CO poisoning, the median serum level of Net-1 was significantly high as compared with controls, meanwhile it was significantly increased after O_2 therapy (6^{th} h) as compared with its level on admission. The present work also found that in cases of chronic CO exposure, the median serum level of Net-1 was significantly increased as compared with controls, with a significant positive correlation between the COHb levels and serum Net-1 levels. The elevated serum levels of Net-1 in cases of either acute or chronic CO exposure were due to its antioxidant and anti-inflammatory effects.

Gedikli et al. (2019) found that serum Net-1 levels were significantly increased in patients with CO poisoning on admission and after O_2 therapy when compared with controls. They also found higher Net-1 levels after O_2 therapy compared to Net-1 levels on admission. They justified their results as despite the clinical improvement and the decrease in blood COHb levels, after O_2 therapy, toxicity and inflammatory status persist at cellular level resulting in high levels of Net-1.

In the present study, there was a significant positive correlation between serum levels of Net-1 and the severity of acute CO poisoning, at 0^{th} h and at 6^{th} h.

Nevertheless, Gedikli et al. (2019) stated that Net-1 was not a useful biomarker for determining the severity of CO poisoning. This can be explained by the fact that the studied CO poisoned cases in their work were mostly consisted of mild cases and they checked the poisoning severity only on bases of neurological involvement.

In the present study, ROC curves were done to compare between serum ADMA, Net-1, and COHb levels as biomarkers in acute CO poisoning, results illustrated that ADMA and Net-1 were more accurate and sensitive biomarkers as compared with COHb. The ROC curves illustrated also that ADMA is more accurate and sensitive biomarker as compared with Net-1.

Veronesi et al. (2017) stated that usage of COHb as a biomarker of acute or chronic CO poisoning is limited by the difficult interpretation of COHb levels. COHb is a very unstable compound and has a short half-life, and therefore, its rapid degradation could generate false negative results leading to underestimation of COHb levels. Conversely, overestimation of COHb levels could occur due to the generation of false positive results in smokers and due to patient comorbidities.

The preference of ADMA as a possible biomarker of CO poisoning can be justified as CO-induced progressive oxidative stress is proved to play an important role in pathophysiology of CO poisoning, either in

initial presentation or after O_2 therapy, and even if COHb blood levels return to normal by O_2 therapy, a high blood level of ADMA, which is an oxidative stress biomarker, must be a sure finding (Yazar et al., 2019).

Conclusion:

Level of COHb alone is an insufficient biomarker for assessing the severity of acute CO poisoning, it can only support the initial diagnosis. In assessing the severity of acute CO poisoning, both ADMA and Net-1 are better biomarkers as compared with COHb.

As a biomarker of acute CO poisoning, ADMA is better than Net-1. In diagnosis of chronic CO poisoning, both ADMA and Net-1 are as dependable as COHb.

Recommendations:

- Usage of ADMA and Net-1 as biomarkers for diagnosis and assessment of the severity of acute CO poisoning, as well as for diagnosis of chronic CO poisoning.
- Conduction of further studies on a large number of patients with acute and chronic CO poisoning in different localities.

Limitations of study:

Small sample size (30 patients with acute CO poisoning): this was due to the low rate of cases of acute CO poisoning admitted to Benha Poisoning Control Unit, plus many of the admitted cases refused to give a consent to participate in the study. In addition, there were limited financial resources.

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Conflict of Interest (if any): 'The Authors declare that there is no conflict of interest'

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نترين-١ و ثنائى ميثيل الأرجنين الغيرمتماثل فى مصل الدم كمؤشرات على التسمم الحاد و المزمن بأول أكسيد الكربون

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يتم تشخيص حالات التسمم بأول أكسيد الكربون بشكل تقليدي عن طريق الكشف عن مستوى الكربوكسي هيموجلوبين، ولكن شدة التسمم الحاد بأول أكسيد الكربون لا علاقة لها بالمستويات المطلقة للكربوكسى هيموجلوبين. نترين-١ هو بروتين له خصائص مضادة للإلتهابات، في حين أن ثنائى ميثيل الأرجنين الغيرمتماثل هو مؤشر إجهاد تأكسدي. لقد هدفت هذه الدراسة إلى تقييم ومقارنة مستويات نترين-١ و ثنائى ميثيل الأرجنين الغيرمتماثل بمصل الدم و الكربوكسي هيموجلوبين كمؤشرات حيوية علي التسمم بأول أكسيد الكربون. أجريت هذه الدراسة السريرية المستعرضة على ٩٠ فردا بعد أخذ الموافقة المستنيرة منهم، على النحو التالى: (٣٠ من البالغين المصابين بالتسمم الحاد بأول أكسيد الكربون الذين تم إدخالهم إلى وحدة علاج التسمم بمستشفيات جامعة بنها؛ ٣٠ عاملاً متعرضين للتسمم المزمنً بأول أكسيد الكربون في أربعة مراكز مختلفة لخدمة السيارات ، بالإضافة إلى ٣٠ من الأشخاص الأصحاء كمجموعة حاكمة). و أظهرت النتائج وجود إرتباط إيجابي بين مستويات نترين-١ و و ثنائى ميثيل الأرجنين الغيرمتماثل فى مصل الدم وشدة التسمم الحاد بأول أكسيد الكربون عند أستقبال الحالات بوحدة علاج التسمم وكذلك بعد العلاج بالأوكسجين، و كان هناك إرتباط إيجابي بين مستوي الكربوكسي هيموجلوبين ومستويات كل من نترين-١ و ثنائى ميثيل الأرجنين الغيرمتماثل فى مصل الدم في حالات التسمم المزمن بأول أكسيد الكربون. و قد أكدت الدراسة الى أن مستوى الكربوكسي هيموجلوبين يدعم فقط التشخيص الأولي للتسمم الحاد بأول أكسيد الكربون، كما خلصت الدراسة الى ان كلا" من ثنائى ميثيل الأرجنين الغيرمتماثل و نترين-١ على الترتيب هما علامات حيوية أفضل في تقييم شدة التسمم الحاد بأول أكسيد الكربون من الكربوكسي هيموجلوبين، وأن كلا" من ثنائى ميثيل الأرجنين الغيرمتماثل و نترين-١ يعتمد عليهم فى تشخيص التسمم المزمن بأول أكسيد الكربون.