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Initial Levels of Serum Neurogranin and Creatine Kinase as Predictors of Delayed Neuropsychiatric Sequelae of Acute Carbon Monoxide Poisoning

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ABSTRACT

Background: Delayed neuropsychiatric sequela (DNS) has been described following carbon monoxide (CO) poisoning. There is a need to identify a new prognostic marker for predicting DNS after acute CO intoxication. This study aimed to investigate the predictive value of initial levels of serum neurogranin (Ng) and creatine kinase-brain (CK-BB) and their role as predictors of DNS in patients with acute CO poisoning.

Methods: This prospective cohort study was conducted on 40 patients of both genders with acute CO poisoning. All participants underwent a comprehensive history taking, clinical examination, and laboratory investigations, including the estimation of serum Ng and CK-BB levels. These cases were followed up for 3 months after acute exposure to CO for DNS signs.

Results: Creatine kinase, CK-BB levels, and plasma Ng levels were significantly higher in DNS-complicated patients, compared to non-complicated ones. Patients with complicated DNS had significantly lower neurological examination, Mini-Mental State Examination (MMSE), and Montreal Cognitive Assessment (MoCA) scores than those with uncomplicated DNS. Based on receiver operating characteristic analysis, COHb, CK-BB, Ng levels, and GCS had cut-off values of >20%, 18.9 U/ L, 10.42 ng/mL, and ≤14, respectively. The sensitivities were 95.65%, 91.3%, 95.65%, and 78.26%, respectively.

Conclusion: Initial serum Ng and CK levels may be helpful predictors of DNS after acute CO poisoning.

Keywords: Carbon monoxide poisoning, Creatine kinase, Delayed neuropsychiatric sequelae, Neurogranin, Predictors

Introduction

Globally, the annual incidence of carbon monoxide (CO) poisoning is estimated to be 137 per million, with a mortality rate of 4.6 per million [1]. Mortality from CO poisoning decreased by 53.5% over the two-decade period due to continued public health education and advances in treatment [2]. CO toxicity arises from tissue hypoxia and direct effects, such as enzyme inhibition (e.g., cytochrome C oxidase). Apoptosis and intracellular oxidative stress are key factors in the pathogenesis of neurotoxicity following CO exposure [1].

The clinical course of CO poisoning may be monophasic or biphasic. The acute manifestations may be either vague, mimicking non-specific viral illnesses, or moderate to severe, resulting in convulsions, cardiovascular collapse, and even death. In the biphasic course, individuals may develop delayed neuropsychiatric sequelae (DNS) [3, 4].

DNS is the most serious complication of CO poisoning. After the acute phase of the poisoning has been successfully treated with medical intervention, DNS may occur in some patients [5]. DNS is characterized by a series of neurological, cognitive, and psychiatric symptoms, including dementia, as well as pyramidal and extrapyramidal dysfunction [3].

Predicting DNS complications should be considered a preventive measure. It is incredibly challenging to identify patients with acute CO poisoning who are likely to develop DNS. The patient's initial presentation does not accurately predict the development of DNS. In addition, there is no screening tool to identify the high-risk group [6].

Creatine kinase (CK) is an intracellular muscle enzyme most often used to diagnose myopathies, including muscle injury. Its concentration in patients with CO poisoning is known to be elevated due to CO-induced ischemia, inflammatory reaction, and low consciousness level [7].

Brain-type CK (CK-BB) is released from the brain into the blood after experimental and clinical brain injury. The presence of a high level of CK-BB in the serum is

associated with a poor neurological prognosis [8].

Neurogranin (Ng) is a protein involved in brain signaling. It is expressed at high levels in the brain. It can be detected in serum or cerebrospinal fluid in conditions that result in damage to brain tissue [9, 10]. CO poisoning causes neurological damage, particularly in the basal ganglia (especially globus pallidus and putamen), thalamus, hippocampus, and corpus callosum, where Ng is abundant [11].

Several studies have investigated tools to predict DNS in patients with CO poisoning; however, there are no standard screening tools or guidelines to predict the development of DNS accurately [7]. Determining accurate predictors for the prognosis of CO poisoning is highly necessary; therefore, this study aimed to investigate the prognostic value of initial serum Ng and CK-BB levels and their role as predictors of DNS in patients with acute CO poisoning.

Materials and Methods

Study design, date, and setting

This prospective cohort study was conducted on patients with acute CO poisoning admitted to the University Poison Control Center. The study was conducted from February 2022 to January 2023.

Ethical considerations

The study received approval from the Ethical Committee of the University Hospitals, Egypt (approval code: 35238/1/22). Written informed consent was obtained from each patient or their legal guardians in the case of unconscious patients. Data confidentiality was maintained by assigning a unique code number to each patient that was only known to the investigators.

Eligibility criteria

Patients of both genders with acute CO poisoning who were presented to the Poison Control Center within 24 hours of exposure were included in this study. Patients were diagnosed based on a history of acute CO exposure, clinical symptoms (e.g., headache, altered mental status, seizures, chest pain, and difficulty in breathing), and elevated COHb levels of > 3-4% in nonsmokers or > 10% in smokers, which confirmed the diagnosis during the initial visit to the Emergency Department.

The diagnosis of DNS was confirmed based on the recurrence of initial neurological or psychological symptoms after recovery periods or the development of new symptoms in patients included in the study.

Exclusion criteria included patients with insufficient clinical and toxicological data to confirm CO poisoning, combined exposure to CO and other intoxicants, and conditions that may lead to a preexisting rise in CK-BB and Ng levels. In addition, patients with a history of substance abuse due to its harmful effect on memory and cognitive function, those with neuropsychiatric disorders that could impact outcome assessment, cases with a history of chronic CO toxicity or prior exposure, those with previous treatment before admission to the Poison Control Center, and the participants who were lost to follow-up were excluded from the study [12].

Methods of the study

All patients were subjected to:

1. History-taking, which includes sociodemographic data (age, gender, residence, education, and occupation), medical history (smoking status, medications, and drug allergies), and toxicological history (the source and duration of CO exposure, delay before medical arrival, and presenting complaints).
2. Clinical assessment upon admission to the hospital involved vital signs, Glasgow Coma Scale evaluation, and examination of the cardiovascular, respiratory, genitourinary systems, and skin. In the current study, severity classification was based on the Olson and Smollin classification [13] and Tomaszewski. [14]. The clinical presentation and COHb level upon admission determined this classification. Mild cases exhibited dizziness and headache with COHb level <20%. Moderate cases presented confusion and syncope with COHb levels between 21% and 40%. Severe cases involved coma and COHb level >40%.
3. Neuropsychiatric follow-up: Patients were given follow-up cards with their follow-up dates scheduled for 1 and 3 months after discharge. Both follow-up visits consisted of a comprehensive neurological examination and cognitive assessment. The Folstein Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) were utilized in the cognitive evaluation. The DNS manifestations included lethargy, emotional instability, personality changes, learning difficulties, psychosis, depression, Parkinsonism, apraxia, gait disorder, urinary incontinence, amnestic syndromes, dementia, and cognitive impairment [15].

Folstein Mini-Mental State Examination (MMSE)

The MMSE has a maximum score of 30 points, with five different domains of cognition analyzed: orientation (up to 10 points), memory (up to 6 points), attention and calculation (up to 5 points), language (up to 8 points), and design copying (up to 1 point). The maximum score on the MMSE is 30; a score of 24 or higher indicates normal cognition. Scores below 24 can suggest cognitive impairment: mild (19–23 points), moderate (10–18 points), and severe (≤9 points) [16].

Montreal Cognitive Assessment (MoCA)

The MoCA is a widely used and concise screening tool for detecting mild cognitive impairment by healthcare professionals. MoCA score ranges are commonly used to assess severity, with scores of 18–25 suggesting mild cognitive impairment, 10–17 indicating moderate impairment, and scores below 10 reflecting severe impairment [17].

4. Laboratory investigations for each patient in this study. A 3 mL arterial blood sample and a 7 mL venous blood sample were withdrawn. The samples were taken immediately after admission and before the administration of any medications under completely aseptic conditions. Laboratory

investigations included a Complete blood count, arterial blood gases, serum sodium (Na), potassium (K) levels, blood glucose, renal functions, alanine aminotransferase, aspartate aminotransferase, CK, CK-BB, and Ng assay. Measurement of CK-BB (U/L) was done by separating the isozymes of CK into well-resolved bands using electrophoresis. "Neurogranin was measured using ELISA kits (Manufacturer: [NEW TEST COMPANY], Germany, Catalog No: [DLR-NRGN-Hu-001). All procedures were performed according to the manufacturer's instructions.

5. Treatment that followed the established Poison Control Center protocol.
6. Assessment of the outcome focused on the development of DNS. Non-complicated patients are those who were discharged from the hospital without any complications and did not develop any neuropsychological sequelae during the follow-up period. On the contrary, DNS-complicated patients refer to patients who had complete recovery of consciousness after an acute CO poisoning event; however, they then developed neuropsychological symptoms within days or months.

Sample size

The Sample size was calculated using Stephen Thompson's equation, with the following parameters: average population (60), error proportion (0.05), probability (50%), and a 95% confidence level (1.96). The sample size consisted of 40 patients.

Statistical analysis

Data were analyzed using IBM SPSS software package (version 20.0) (Armonk, NY: IBM Corp). Qualitative data were described using numbers and percentages. The Shapiro-Wilk test was used to verify the normality of the distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median, and interquartile range.

Chi-square test, Fisher's Exact, or Monte Carlo correction for Chi-square were used for categorical variables. The Student t-test was used for normally distributed quantitative variables, and the Mann-Whitney test was used for abnormally distributed quantitative variables.

The receiver operating characteristic (ROC) curve was generated from the data to determine the optimal cut-off point, sensitivity, and specificity. The area under the curve (AUC) indicates the diagnostic performance of the test. It was graded as follows: 0.90-1=excellent; 0.80-0.90=good; 0.70-0.80=fair; and 0.60- 0.70=poor. Pairwise comparisons of the AUCs of the studied parameters were made. A P-value <0.05 indicated significance in interpreting the results of statistical tests.

Results

During the study period, 55 patients with acute CO poisoning were admitted to the Poison Control Center. They were screened for eligibility. Only 40 patients were ultimately enrolled in the study. The remaining 15 were excluded for various reasons: met exclusion criteria (n=6), undetermined storage errors (n=4), died during hospital stay (n=2), and refused follow-up (n=3).

Table 1 summarizes the characteristics of the studied patients with acute CO poisoning. Their ages ranged from 14 to 58 years. Men outnumbered women, with a median exposure duration of 1 hour. The source of exposure was a gas water heater in 55% of cases. Clinical manifestations and COHb levels indicated that 42.5% of cases had mild poisoning, while moderate grades were found in 45%, and severe grades in 12.5% of cases. Serum Ng levels ranged from 5.03 ng/dL to 26.82 ng/dL, and serum CK-BB levels ranged from 6.4 U/L to 4580 U/L. Elevated serum CK levels were found in 57.5% of patients. Delayed neuropsychiatric symptoms developed in 57.7% of patients within the first three months post-exposure (DNS complicated group). In contrast, 42.5% of patients didn't develop DNS (non-complicated group). The frequency of detected symptoms within DNS complicated patients was memory loss (53.2%), concentration deficit (34.8%), and vegetative state (4.3%) (data is not tabulated).

Table 1. Baseline characteristics of patients with acute carbon monoxide poisoning

		N=40
Age (years)		27.33±10.36
Gender	Male	21(52.5%)
	Female	19(47.5%)
Delay (hours)		3.0(2.0–4.0)
Duration of exposure (hours)		1.0(0.50–2.0)
Source of CO	Domestic butane	2(5.0%)
	Fire	5(12.5%)
	Gas water heater	22(55.0%)
	Charcoal burns in closed	11(27.5%)
COHb level (%)		24.50(17.5–35.0)
Severity of acute CO poisoning	Mild (15-20)	17(42.5%)
	Moderate (21-40)	18(45.0%)
	Severe (41-59)	5(12.5%)
Ng (ng/dl)		12.82±4.80
CKBB (U/L)		22.20(15.50–42.10)
CK	Normal	23(57.5%)
	Elevated	17(42.5%)
DNS	Yes	23(57.5%)
	No	17(42.5%)

Data is presented as mean ± SD or frequency (%) or median (IQR). CO: carbon monoxide, Ng: Neurogranin, COHb: carboxyhemoglobin, CK: creatine kinase; CK-BB: creatine kinase brain type, DNS: delayed neuropsychiatric sequelae.

According to the appearance of DNS after co-poisoning, acute CO-poisoned patients were divided into DNS-complicated and non-complicated patients.

The study found significant differences between the two patient groups in terms of age, gender, occupation, and special habits. On the contrary, no significant differences were found in other sociodemographic data. The two groups were comparable in terms of source of exposure and delay time; however, the DNS-complicated group had a significantly longer median duration of CO exposure. Upon admission, there were no significant differences in symptoms of CO poisoning, except for syncope, as shown in Table 2.

Table 2. Comparison between the DNS complicated group and the non-complicated group regarding sociodemographic data, source of CO, delay time, duration of exposure, and presenting symptoms

		DNS (n = 23)	Non-DNS (n = 17)	Test (χ2)	P
Age (years)		31.17±11.23	22.12±6.16	t=3.260*	0.002*
Gender	Male	19(82.6%)	2(11.8%)	19.673*	<0.001*
	Female	4(17.4%)	15(88.2%)		
Marital Status	Single	10(43.5%)	9(52.9%)	0.351	0.554
	Married	13(56.5%)	8(47.1%)		
Residence	Rural	17(73.9%)	10(58.8%)	1.015	0.314
	Urban	6(26.1%)	7(41.2%)		
Education	Read and write+ 1ry, perp	7(30.4%)	4(23.5%)	1.154	0.562
	Secondary school	7(30.4%)	8(47.1%)		
	University and high institutes	9(39.1%)	5(29.4%)		
Occupation	Manual worker	9(39.1%)	0(0.0%)	13.116*	MCP= 0.004*
	Housewife	3(13.0%)	7(41.2%)		
	Students	5(21.7%)	8(47.1%)		
	Employed	6(26.1%)	2(11.8%)		
Special habit	Smoking	8(34.8%)	0(0.0%)	7.391*	FEP= 0.013*
	No	15(65.2%)	17(100.0%)		
Source of CO	Domestic butane	2(8.7%)	0(0.0%)	3.452	MCP=0.374
	Fire	4(17.4%)	1(5.9%)		
	Gas water heater	10(43.5%)	12(70.6%)		
	Charcoal burn in closed	%) 7(30.4	4(23.5%)		
Delay (hours)		3.50(2.0– 6.25)	2.0(2.0–3.0)	U=143.0	MCP=0.156
Duration of exposure (hours)		1.0(1.0–3.25)	0.50(0.50–0.50)	U=94.50*	MCP=0.005*
Symptoms	Syncope	21(91.3%)	10(58.8%)	5.914*	FEP=0.023*
	Dizziness	22(95.7%)	13(76.5%)	3.288	FEP=0.144
	Vomiting	15(65.2%)	9(52.9%)	0.614	0.433
	Headache	9(39.1%)	10(58.8%)	1.520	0.218
	Seizures	5(21.7%)	0(0.0%)	4.224	FEP=0.061
	Urination/defecation	%) 5(21.7	1(5.9%)	6.625	MCP= 0.052
	Cyanosis	4(17.4%)	0(0.0%)		
	Compartments	1(4.3%)	0(0.0%)		

Data are presented as mean ± SD or frequency (%) or median (IQR). *Significant p value <0.05. DNS: Delayed neurological sequalae, CO: Carbon monoxide, X^2: Chi square test; MC: Monte Carlo; FE: Fisher Exact, U: Mann Whitney test.

The clinical examination revealed significant statistical differences in diastolic blood pressure (DBP), GCS, and respiratory system evaluation between the two groups (P≤0.05). Patients were categorized based on the degree of impairment in their consciousness. There was a significant difference between the two groups regarding impairment in consciousness levels (P≤0.05). The laboratory investigations revealed that patients with DNS complications had significantly lower median HCO_3 levels, mean $PaCO_2$ levels, and SO_2 compared to non-complicated patients. However, all laboratory tests, including Hb levels, blood cell counts, liver enzymes, and kidney function markers, were within normal values (data are not tabulated). The study found that non-invasive COHb, CK, CK-BB, and serum Ng levels were significantly higher in patients with DNS complications, compared to those without complications (P<0.05), as shown in Table 3.

Table 3. Comparison between the DNS complicated group and the non-complicated group as regards vital signs, consciousness level assessment, chest examination, ABG, level of COHb, levels of CK, CK-BB, and serum Ng level at admission

		DNS (n = 23)	Non-DNS (n = 17)	Test	P
Vital signs	SBP (mmHg)	107.8±17.83	117.6±10.91	t=2.007	0.052
	DBP (mmHg)	69.35±14.17	79.41±10.88	t=2.442*	0.019*
	HR (beat /minute)	95.83±24.42	89.18±14.64	t=1.071	0.291
	RR (cycle/minute)	18.0(18.0–20.0)	18.0(18.0–20.0)	U=187.0	0.829
	Temperature (°C)	36.94±0.24	37.0±0.0	t=1.153	0.261
GCS		14.0(8.50–14.0)	15.0(14. 0–15.0)	U=91.50*	0.004*
Level of consciousness	Minor (≥13)	16(60.8%)	5(94.1%)	χ^2=9.490*	MCP= 0.018*
	Moderate (9-12)	3(13.0%)	1(5.9%)		
	Severe (≤8)	6(26.1%)	0(0.0%)		
Chest	Normal	17(73.9%)	17(100.0%)	χ^2=5.217*	FEP= 0.030*
	Bilateral crepitation	6(26.1%)	0(0.0%)		
ABG	pH	7.41±0.07	7.42±0.05	t=0.907	0.370
	$PaCO_2$ (mmHg)	30.08±7.20	37.88±8.58	t=3.124	0.003*

	HCO_3 (mEq/L) PaO_2 (mmHg) SO_2(%)	18.0(17.35–22.65) 88.0(81.0–90.0) 92.91±6.18	22.0(21.0–24.0) 88.0(82.0–89.0) 96.94±3.07	U=108.0* U=180.0 t=2.707*	0.016* 0.685 0.011*
COHb (%)		34.0(28.5–38.5)	15.0(10.0–20.0)	U=5.50*	<0.001*
CK (U/L)	Normal Elevated	6(26.1%) 17(73.9%)	17(100.0%) 0(0.0%)	χ^2=21.853*	<0.001*
CKBB (U/L)		40.80(32.0–84.30)	14.0(12.30–17.50)	U=9.500*	<0.001*
Ng (ng/mL)		15.86±3.87	8.71±2.11	t=7.488*	<0.001*

Data are presented as mean ± SD or frequency (%) or median (IQR). *Significant p value <0.05. DNS: Delayed neuropsychiatric sequalae, COHb: Carboxyhemoglobin, ABG: Arterial blood gases, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, HR: Heart rate, RR: Respiratory rate, GCS: Glasgow coma scale, PCO_2: partial pressure of carbon dioxide; PaO_2: partial pressure of oxygen; HCO_3: bicarbonate; pH: potential of hydrogen, SO_2: Oxygen saturation, CK: Creatine kinase, CKBB: Creatine kinase brain type, Ng: Neurogranin level.

The severity of acute CO poisoning at presentation was significantly higher in DNS-complicated patients, compared to non-complicated ones, and the number of HBO sessions was higher in DNS-complicated patients. During follow-up, significant differences were found in neurological examination, median MMSE, and median MoCA test parameters between the two groups (Table 4).

Table 4. Comparison between the DNS complicated group and the non-complicated group regarding the severity of acute CO poisoning, number of HBO sessions, and follow-up visits at one and three months

		DNS (n = 23)	Non-DNS (n = 17)	Test	P
Severity of acute CO poisoning	Mild Moderate Severe	1(4.3%) 17(73.9%) 5(21.7%)	16(94.1%) 1(5.9%) 0(0.0%)	χ^2 34.469*	<0.001*
HBO therapy		18(78.3%)	12(70.6%)	0.307	FEP= 0.717
Number of HBO sessions		3 (2.0 – 5.0)	1.0 (1.0 – 1.50)	U=46.50*	0.008*
		Follow-up visit at one month			
Neurological examination	Normal Abnormal	16(69.6%) 7(30.4%)	17(100.0%) 0(0.0%)	χ^2=6.271*	0.014*
MMSE test		22.0 (19.0 – 23.0)	28.0 (26.0 – 28.0)	U=7.500*	<0.001*
MoCA test		20.0 (17.50 – 22.0)	26.0 (26.0 – 27.0)	U=17.000*	<0.001*
		Follow-up visit at three months			
Neurological examination	Normal Abnormal	17(73.9%) 6(26.1%)	17(100.0%) 0(0.0%)	χ^2=5.217*	0.030*
MMSE test		25.0 (20.0 – 26.0)	29.0 (28.0 – 29.0)	U=23.500*	<0.001*
MoCA test		23.0 (19.50 – 25.0)	28.0 (27.0 – 28.0)	U=19.500*	<0.001*

Data are presented as mean ± SD or frequency (%) or median (IQR). *Significant p value <0.05. CO: Carbon monoxide, HBO: Hyperbaric oxygen, MMSE: Mini-Mental Examination, MoCA: Montreal Cognitive Assessment score.

Pearson correlation analysis revealed a strong positive correlation between Ng and memory loss (r = 0.78, P<0.001), while only weak to moderate correlations were observed between Ng and other DNS symptoms. CK-BB showed weak, mostly non-significant correlations with the studied symptoms (Table 6).

Table 5. Performance of COHb, serum Ng, serum CK-BB levels, and GCS for prediction of development of DNS in patients with acute carbon monoxide poisoning

	COHb	CK-BB	Ng	GCS
AUC	0.986	0.976	0.962	0.766
P	<0.001*	<0.001*	<0.001*	0.004*
95% CI	0.959 – 1.0	0.937 – 1.0	0.902 – 1.0	0.620 – 0.912
Cut-off	>20	>18.9	>10.42	≤14
Sensitivity	95.65	91.30	95.65	78.26
Specificity	94.12	88.24	88.24	64.71
PPV	95.7	91.30	91.7	75.0
NPV	94.1	88.24	93.8	68.7
P value from pairwise comparison of AUCs				
COHb	------	0.624	0.388	0.002*
CKBB	0.624	------	0.673	0.003*
Ng	0.388	0.673	------	0.003*
GCS	0.002*	0.003*	0.003*	------

AUC: area under the curve, CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value. * Significant P value<0.05. COHb: carboxyhemoglobin, Ng: Neurogranin, CK-BB: creatine kinase brain type, DNS: delayed neuropsychiatric sequelae, GCS: Glasgow coma scale.

Table 6. Correlation between Biomarkers (CKBB & Ng) and DNS Symptoms in patients with acute carbon monoxide poisoning.

Symptom / Biomarker	Ng (r, p)	CK-BB (r, p)
Headache	0.22 (p>0.05)	-0.11 (p>0.05)
Insomnia	0.30 (p>0.05)	0.15 (p>0.05)
Memory loss	0.78 (p<0.001) *	0.28 (p>0.05)
Concentration deficit	0.45 (p>0.05)	0.20 (p>0.05)

Ng: Neurogranin, CK-BB: creatine kinase brain type, DNS: delayed neuropsychiatric sequelae, *Significant p value <0.05, r: Pearson correlation coefficient

ROC curves were conducted to assess the accuracy of the studied markers (CK-BB & Ng) and for GCS and

COHb in predicting DNS in patients with acute CO poisoning. Initial CK-BB levels had a sensitivity of 91.3% and a specificity of 88.24%, while initial Ng levels had a sensitivity of 95.6% and a specificity of 88.24%. COHb showed a sensitivity of 95.60% and a specificity of 94.12%, while GCS had a sensitivity of 78.26% and a specificity of 64.71%. Carboxy haemoglobin had the best area under the curve (AUC), followed by CKBB, Ng, and GCS. Pairwise comparisons revealed no significant differences between all predictors, except for GCS, which showed a substantial difference with all other parameters (Table 5 and Figure 1).

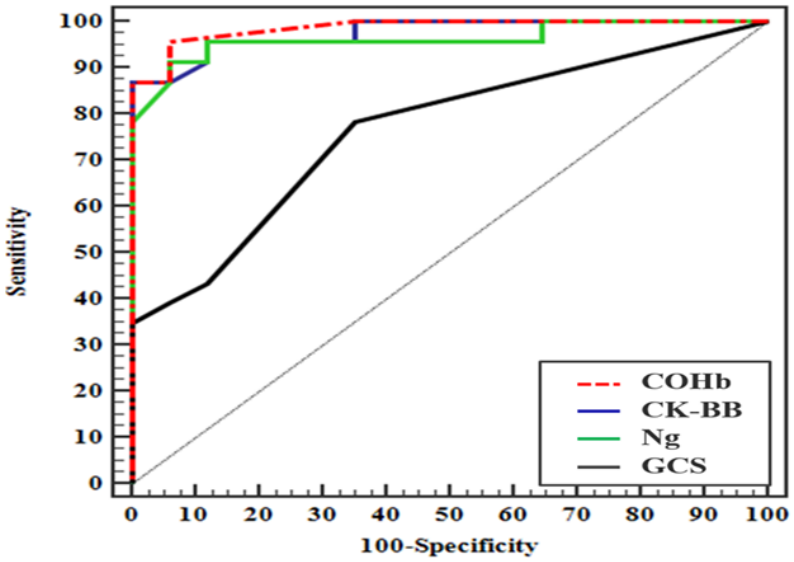

Figure 1. ROC curve for prediction of development of delayed neurological sequalae in patients with acute carbon monoxide poisoning using neurogranin level, creatine kinase brain type, Glasgow coma scale, and carboxyhemoglobin

Discussion

Preventing DNS is a crucial goal in treating CO poisoning; however, predicting risk factors is challenging, with limited clinical utility [18]. This study aimed to assess the prognostic value of initial serum Ng and CK levels in predicting DNS in patients with acute CO poisoning.

The study found that 57.5% of CO-intoxicated patients developed DNS. This was consistent with previous studies by Helal et al. [19] and Shahin et al. [20]. On the contrary, Lee et al. [7] contradicted the current study by reporting that only 8.7% of cases developed DNS. Caballero-Bermejo et al. [21] suggested that discrepancies in DNS incidence among studies are due to a lack of uniformity in follow-up visits between studies. Additionally, Lee et al. [7] noted that there is no established diagnostic guideline for DNS, leading to varying criteria for defining patients. Other factors, such as population, patient age, CO exposure duration, delay time, and gas concentration during exposure, may contribute to discrepancies [22].

The study found significant differences in age and gender among patients with and without DNS, supporting Zhang et al. [1] suggestion that older age is a risk factor for DNS. On the other hand, Gaballah et al. [23] found similar baseline characteristics, including age and gender, in patients with and without DNS.

In the current study, the DNS-complicated group had significantly more prolonged exposure to CO gas, compared to the non-complicated group. Huang et al. [3] established that the brain possesses high sensitivity to hypoxia because of its high metabolic rate. Therefore, a longer duration of exposure to CO would allow more O_2 deprivation to the brain and a higher CO load in tissues and Hb, increasing susceptibility to CNS insults and DNS [1].

The study found no significant difference in the median prehospital delay period between DNS-complicated and non-complicated patients, consistent with Gao et al. [18], who also found no significant difference.

The current study found that syncope was more frequent in the DNS-complicated group than in the non-complicated group, similar to the finding reported by Mubarak et al. [24].

The mean SBP and DBP in the DNS complicated group were lower than those in the non-complicated group; however, the difference was only significant for the mean DBP. Ghanem et al. [25] reported that the mean SBP and DBP were significantly lower in the DNS group. Lee et al. [7] stated that low DBP leads to hypoxia, decreased oxygen saturation, and cerebral hypoperfusion. This results in vascular changes, damage to the blood-brain barrier, and increased vascular permeability. This results in the accumulation of amyloid protein in the cerebral parenchyma, leading to neuronal death.

The study found that the initial GCS in the DNS-complicated group was significantly lower than in the non-complicated group. Pepe et al. [26] found that longer exposure times and GCS values less than 9 were correlated with the development and severity of DNS following CO poisoning.

This study found that a significantly higher percentage of patients in the DNS-complicated group had bilateral basal chest crepitations, compared to the non-complicated group. This finding could exacerbate

hypoxemia, potentially increasing the occurrence of DNS. Mubarak et al. [24] support this finding, noting a statistically significantly higher percentage of bilateral chest crepitations in the DNS group, compared to the non-DNS group.

Laboratory investigations revealed a significantly lower mean $PaCO_2$ value in the DNS-complicated group, compared to the non-complicated group. Ashry et al. [27] found that hypocapnia can lead to cerebral vasoconstriction, reducing brain blood flow, which can exacerbate the effects of hypoxia on the brain. The study also observed a significantly lower median of HCO_3 levels in the DNS complicated group, consistent with Ghanem et al. [25]. Additionally, the mean SO_2 of DNS-complicated patients was significantly lower than that of non-complicated patients. Shahin et al. [20] found a similar difference in mean SO_2 upon admission.

The study found that patients with DNS-complicated conditions had significantly higher mean COHb levels than those without. This finding is consistent with previous research by Helal et al. [19] and Ghanem et al. [25] who also found a higher mean COHb level in the DNS group.

The study found that the DNS-complicated group had significantly higher mean plasma levels of Ng within 24 hours, compared to the non-complicated group, and both groups had higher levels than the health control group reported in other studies. Among healthy control subjects, Yang et al. [28] found Ng levels to be 0.002 ng/mL, while Yeşilyurt et al. [11] reported Ng was 0.22 ng/mL. The high level of Ng observed in the current study among patients with CO poisoning could be attributed to the small size of Ng, which can pass through damaged blood-brain barriers. Furthermore, Ng was significantly higher in the DNS complicated group because of the neuronal injury induced by CO poisoning [28]. There are two mechanisms for such neuronal injury: (1) Cellular hypoxia (2) Inflammation through independent pathways, such as post-ischemic reperfusion injury, vascular endothelium affected by CO, and oxygen radical-mediated lipid peroxidation. Based on this explanation, it goes to say that patients in the DNS complicated group would have more neuronal cell damage and consequently higher Ng levels compared to those in the non-DNS group [29].

The total CK was significantly elevated in a higher percentage of patients in the DNS complicated group. These findings are consistent with the results of Gaballah et al. [23] Additionally, Kitamoto et al. [30] concluded that an elevated CK level may be linked to the development of DNS. This increase indicates both CO-mediated muscle necrosis and rhabdomyolysis in comatose patients who have been lying on flat surfaces for extended periods.

The DNS group showed significantly higher initial serum CK-BB levels than the non-complicated group. To the best of the authors' knowledge, there are no studies in the literature that analyzed the level of CK-BB in cases of acute CO poisoning. The CK phosphagen system is fundamental to cellular energy homeostasis. The level of CK-BB has been found to be a marker of brain injury and is elevated after exposure to hypoxic conditions [31].

In the present study, a significant difference between the DNS and non-DNS complicated groups was detected in terms of the level of severity of CO poisoning. Ning et al. [32] reported that the clinical characteristics of CO poisoning and COHb level are so reliable that they can be used to predict DNS after CO poisoning. Severe cases showed a high risk for the development of DNS or neurological sequelae. Moreover, moderate or severe degrees of severity can trigger pathophysiologic processes that result in brain damage and morbidity, which differ from mild cases.

In this study, patients with DNS complications required a significantly higher number of HBO sessions, compared to those without complications. These findings are consistent with the results of Helal et al. [19] patients with DNS require more HBO sessions due to prolonged exposure to CO gas, lower consciousness, lower SO_2, and higher COHb levels, resulting in a more severe presentation.

Patients with DNS showed significantly lower MMSE and MoCA scores at 1 and 3 months post-exposure. Wu et al. [33] also found lower scores in patients with delayed encephalopathy after CO poisoning compared to healthy controls.

ROP curves were used to assess the accuracy of markers, such as CK-BB & Ng, GCS, and COHb levels, in predicting DNS in CO poisoning patients. The COHb level showed the best discriminatory power, with a sensitivity of 95.65% and specificity of 94.12%. This finding aligns with the findings of Mubarak et al. [24] and Ghanem et al. [25] studies. They indicated that COHb levels have excellent predictive power for DNS.

To the best of the author's knowledge, this is the first study to use serum Ng and CK-BB levels for the prediction of DS in patients with acute CO poisoning. The level of CK-BB had the second-highest AUC of 0.976 at a cut-off value greater than 18.9 U/L with a sensitivity of 91.3% and specificity of 88.24%. The CK-BB levels have been used previously for detecting brain damage caused by hypoxia. Alkholy et al. [31] discovered that cord blood CK-BB levels are a good early indicator of severe hypoxic ischemic encephalopathy in newborn infants.

Serum NG level has an AUC of 0.962 for predicting DNS at a cut-off value of>10.42 ng/mL, with a sensitivity of 95.65% and a specificity of 88.24%. Neurogranin was used before for the detection or prediction of neurological and mental diseases. It was used for detecting patients who have pathological CT findings of mild TBI. In a study conducted by Çevik et al. [34] it had an AUC of 0.767 at a cut-off level of 1.87ng/mL. Yeşilyurt et al. [11] concluded that Ng may be a new diagnostic biomarker for CO poisoning.

The GCS had the lowest predictive power with an AUC of 0.766 at a cut-off of 14 or lower. The sensitivity was 78.26% and the specificity was 64.71%. Ghanem et al. [25] determined that GCS had good discriminatory power for predicting DNS (AUC of 0.691 at a cut-off value ≤13).

The use of serum Ng and CK levels as predictors of DNS has a significant clinical implication, enabling

early intervention and targeted therapeutic strategies in emergency settings. This could potentially improve patient outcomes and reduce the long-term consequences of CO poisoning.

Limitations of the study include a relatively small sample size, limiting the generalizability of our findings. In addition, the study was conducted at a single center, and multicenter studies are necessary to validate our results. The observational nature of the study also limits the ability to determine causality. The three-month follow-up is relatively short for studying DNS, which may manifest later. A longer follow-up period would provide insights into long-term outcomes. This study did not collect or analyze detailed data on therapeutic interventions administered to the patients. Therefore, the potential effects of treatment modalities on Ng and CK-BB levels could not be assessed. This represents a limitation of our work. Future research should investigate how different therapeutic interventions for poisoning may influence these biomarkers.

Conclusions

Initial serum Ng and CK levels are promising predictors of DNS in patients with acute CO poisoning. These findings emphasize the potential of these biomarkers to enhance the prognosis and management of CO poisoning.

Conflict of Interests

The authors have no financial or proprietary interest in any material discussed in this article.

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Ethical Considerations

This study was conducted in accordance with the principles of ethics. The study received approval from the Ethical Committee of Tanta University Hospitals in Tanta, Egypt (approval code: 35238/1/22).

Written informed consent was obtained from each patient or their legal guardians in the case of unconscious patients

Authors' Contributions

All authors contributed to the conception and design of the study. Material preparation, data collection, and analysis were performed by [M. M. E.], [B. H. F.] and [A. A. W.]. The first draft of the manuscript was written by [A. A. E.] and [M. S. E.]. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. All figures of the manuscript were original.

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