The Possible Role of Neuron Specific Enolase and Neurofilament Light Protein as Markers for Organophosphorus-induced Neurotoxicity

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Abstract

Background: Acute organophosphorus (OP) poisoning is a common toxic emergency all over the world especially in the developing countries as Egypt. Neurological damage occurs after exposure to these compounds can lead to respiratory failure and death. Thus, it is important to identify the severity of poisoning and predict the need to ventilation support or death.

Methods: A prospective study that included 50 adult patients presented with neurological manifestations after acute OP poisoning admitted to the ICU of Poison Control Center of Ain shams university (PCC-ASU), and 25 healthy volunteers. Meusering levels of Neuron Specific Enolase (NSE) and Neurofilament Light (NFL) protein and correlate this with severity according to APACHE II score and outcome of patients.

Conclusion: There was a significant difference between cases and controls among levels of NSE and NFL protein, also there was a significant correlation between NSE and NFL protein levels and prediction of both M.V. need and mortality, so NSE and NFL protein can be used as markers for neurological damage after exposure to OP compounds.

Key Words: *APACHE II score, Neurofilament Light Protein, Neuron Specific Enolase, Neurotoxicity and Organophosphorus.*

Introduction

Organophosphorus (OP) compounds are the most commonly used insecticides to control agricultural and household pests. The easy availability with lack of knowledge about its serious consequences resulting in increased its accidental and suicidal poisoning [1].

Exposure to (OP) compounds leads to inhibition of cholinesterase enzyme with subsequent accumulation of acetylcholine at synapses causing overstimulation of muscarinic and nicotinic receptors leading to central and peripheral manifestations [2].

Many patients, following acute exposure to these compounds, will develop muscle weakness and paralysis especially in severe exposures, and patients will require prolonged ventillatory support in the intensive care unit and patients die because of respiratory failure. The neurological manifestations have therefore been a primary focus of interest [3].

Neuron Specific Enolase (NSE) is measured in blood and cerebrospinal fluid is predominantly located in neurons and neuroectodermal cells so it serves as a marker of neuronal damage. Increased concentration of NSE can be measured in the cerebrospinal fluid (CSF) and in peripheral blood after neuronal damage and provides a reliable laboratory indicator of the degree of brain cell damage [4].

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Neurofilaments are cytoskeletal components of neurons that are abundant in axons particularly in the long myelinated subcortical white matter axons. The release of NFL sharply increases in response to CNS axonal damage because of inflammatory, neurodegenerative, traumatic or vascular injury. The NFL that is released reaches the interstitial fluid, which communicates freely with the CSF, and blood [5].

Patients and Methods

This work presents a prospective study carried out at Poison Control Center of Ain Shams University (PCC-ASU), cairo, Egypt, during the period from February 2019 till June 2020.

Patients:

The study included 50 adult patients with acute organophosphorus poisoning admitted to the ICU of Poison Control Center of Ain shams university hospitals (PCC-ASUH), and 25 healthy volunteers.

Inclusion criteria

All patients with acute organophosphorus poisoning admitted in ICU of (PCC-ASU) and presented with any neurological manifestations.

Exclusion criteria

Patients with head trauma, patients with stroke or brain hemorrhage, patients with any neurological diseases, addicts and patients who taken any drugs known to affect the CNS were excluded from the study.

Methods

History taking

Full history including sociodemographic data; (age, gender and residence), intoxication history including manner of exposure to OP (either suicidal, accidental or homicidal), route of exposure (either oral, dermal, Inhalation or injection), delay time (time interval between exposure and arrival to the PCC) and history of co-ingestion.

Clinical examination

All patients were clinically examined regarding characteristic symptoms and signs of cholinergic toxidrome which include muscarinic manifestations, nicotinic manifestations and CNS effects.

Neurological examination was done for each patient including conscious level assessment according to Reed's classification, recording presence of fasciculation, muscle weakness, flaccid paralysis, coma, agitation, convulsions, motor or sensory deficit, reflexes and any lateralization signs will be recorded.

Evaluation of patients with APACHE II score [6]. The APACHE II score was calculated using designed computerized program during first 24 hours of admission. If a variable was measured more than once during that time, the worse value was used.

Laboratory data

1. Patients were be subjected to the following biomarkers; Neuron Specific Enolase (NSE) and Neurofilament Light protein (NFL) on presentation to ER within 8 hours after OP exposure due to short half-lives of these biomarkers also pseudocholinestrase (PChE) was done to confirm diagnosis of OP toxicity.

Laboratory parameters for neurological damage; Neuron Specific Enolase (NSE) and Neurofilament Light Protein (NFL) were done by Enzyme-Linked Immunosorbent Assay (ELISA) technique using commercial ELISA Kit from Bioassay Technology Laboratory company following the manufacturer instructions.

2. Patients were be subjected also to the following routine parameters within first 24 hours of ICU admission for calculation of APACHE II score:

Serum glucose, kidney function tests; (urea and creatinine), serum electrolytes; (sodium and potassium), CBC and ABG.

3. Controls were be subjected to the following biomarkers; NSE, NFL and also PChE.

Treatment and hospital disposition:

Documentation of total dose of atropine and oximes received, duration of stay in the PCC and outcome (either discharge or death).

Statistical Analysis

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 20). Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

Mean, standard deviation (±SD) was done for numerical data. Frequency and percentage were obtained for non-numerical data. Comparison between outcome groups was tested by using Independent t-test for quantitative data, by using Chi square test for qualitative data. Correlation analysis (using Pearson's method) was used to assess the strength of association between two quantitative variables. Linear regression analysis was used to identify significant predictors of outcomes. P-value less than 0.05 was considered statistically significant. Receiver operating characteristic curve (ROC) was used to assess predictors of outcome with its cut off points, sensitivity, specificity, positive Predictive Value and negative Predictive Value.

Results

The study showed a non significant difference between cases and controls regarding their gender, residence or age as shown in table (1),

Our study showed that the suicidal exposure to OPCs was the most common manner of exposure (78%), while oral route was the most common route of exposure (86%). Regarding delay time, the mean delay time for cases was 5.1 ± 2.3 hours as shown in table (2).

On assessing conscious level of cases by Reed's classification, most of patients were comatose in grade III and grade IV by 30% for each grade and 12% of patients were conscious (grade 0). Regarding neurological manifestations, most of patients were presented with weakness, fasciculation and paralysis with a percentage of 72%, 68% and 50% respectively as shown in table (3).

The study showed that there was a significant difference between cases and controls regarding Pseudocholinestrase (PChE), Neuron Spacific Enolase (NSE) and Neurofilament Light (NFL) Protein levels as shown in table (4).

There was a significant positive correlation between NSE and NFL Protein levels with conscious level, also there was a significant positive correlation between NSE level and fasciculation and agitation, and there was a significant negative correlation with paralysis and convulsions. There was a significant positive correlation between NFL Protein level and fasciculation and there was a significant negative correlation with paralysis as shown in table (5).

The study showed that the mean APACHE II score for cases was 14.3 ± 8.3 and its range was between 3 and 28. There was a significant correlation between APACHE II score and M.V. need and death outcome as showed in table (6)

There was a significant positive correlation between NSE and NFL Protein levels with severity according to APACHE II score, total atropine dose and hospital stay duration, as shown in table (7)

The study showed that there was a significant positive correlation between NSE and NFL Protein levels and need to M.V., as shown in figure (1) also there was a significant positive correlation between NSE and NFL protein levels with mortality outcome as shown in figure (2), which means that high levels of these parameters associated with more need to M.V. and more deaths.

By ROC curve analysis to predict M.V. need and mortality, it was found that NSE had a greater sensitivity and specificity than NFL protein level in prediction M.V. need and mortality as shown in table (8) and table (9).

Table (1): Gender, Residence and age differences between controls and cases

N (%)		Controls	Cases	Chi-Square	
		N (%)	X^2	p value	
Gender	Male	14 (56.0%)	24 (48.0%)	0.43	0.514 **(NS)**
	Female	11 (44.0%)	26 (52.0%)		
Residence	**Urban**	11 (44.0%)	23 (46.0%)	0.03	0.870 **(NS)**
	Rural	14 (56.0%)	27 (54.0%)		
Mean ± SD				Student T test	
		Mean ± SD	t value	P value	
Age		34.2 ± 11.5	32.3 ± 12.6	0.65	> 0.05 **(NS)**

N: Number %: Percentage

X^2: chi square T: student t test

P value >0.05: is considered non significant (NS)

Table (2): Manner and Route of exposure to OPCs

		N (%)
Manner of exposure	Accidental	11 (22.0%)
	Suicidal	39 (78.0%)
Route of exposure	Oral	43 (86.0%)
	Inhalation	5 (10.0%)
	Injection	1 (2.0%)
	Dermal	1 (2.0%)
	Mean ± SD	
Delay time (hours)	5.1 ± 2.3	

N: Number %: Percentage SD: standard deviation

Table (3): Clinical manifestations of cases

		N (%)
Conscious level	Grade 0 (conscious)	6 (12.0%)
	Grade I	8 (16.0%)
	Grade II	6 (12.0%)
	Grade III	15 (30.0%)
	Grade IV	15 (30.0%)
Neurological manifestations	Fasciculation	34 (68.0%)
	Weakness	36 (72.0%)
	Paralysis	25 (50.0%)
	Agitation	11 (22.0%)
	Convulsions	9 (18.0%)

N: Number %: Percentage

Table (4): Differences between controls and cases in Pseudocholiestrase, Neuron Spacific Enolase and Neurofilament Light Protein levels

	Controls	Cases	Student's T test	
	Mean ± SD	Mean ± SD	t value	p value
PChE (U/L)	7580.9 ± 1613.2	1096.6 ± 500.1	19.63	<0.001(S)
NSE (ng/ml)	1.91 ± .63	26.05 ± 11.48	-14.82	<0.001(S)
NFL (ng/ml)	2.88 ± .77	22.75 ± 9.68	-14.43	<0.001(S)

SD= standard deviation t: student's T test

P: < 0.05 significant (S)

Table (5): Clinical manifestations in relation to NSE and NFL levels

Student's T test					
		NSE (ng/ml)	**t (p value)**	**NFL (ng/ml)**	**t (p value)**
Fasciculation	No (N =16)	33.8 ± 10.14	t =3.67 p = 0.001 (S)	26.66 ± 8.72	t =2.02 p = 0.049 (S)
	Yes (N =34)	22.4 ± 10.3		20.91 ± 9.67	
Weakness	No (N =14)	23.86 ± 12.94	t =-0.84 p = 0.405 (NS)	22.36 ± 10.66	t =-0.18 p = 0.86 (NS)
	Yes (N =36)	26.9 ± 10.94		22.9 ± 9.42	
Paralysis	No (N =25)	18.62 ± 8.49	t =-5.99 p = <0.001 (S)	19.19 ± 9.96	t =-2.77 p = 0.008 (S)
	Yes (N =25)	33.48 ± 9.05		26.31 ± 8.09	
Agitation	No (N =39)	27.81 ± 11.32	t =2.11 p = 0.04 (S)	22.79 ± 9.48	t =0.05 p = 0.958 (NS)
	Yes (N =11)	19.82 ± 10.19		22.61 ± 10.81	
Convulsions	No (N =41)	23.88 ± 10.99	t =-3.08 p = 0.003 (S)	21.69 ± 9.75	t =-1.69 p = 0.098 (NS)
	Yes (N =9)	35.92 ± 8.35		27.58 ± 8.14	
	Sperman correlation				
Conscious level	r	0.768		0.457	
	p value	<0.001 (S)		0.001 (S)	

t: student's t test

r: coefficient of correlation

P: < 0.05 significant (S) P: > 0.05 non significant (NS)

Table (6): APACHE II Score in relation to M.V. need and outcome

	M.V. need		**t test**	
	No	**Yes**		
	Mean ± SD	Mean ± SD	t	p value
APACHE II Score	4.7 ± 1.8	19.2 ± 5.5	-10.59	<0.001 (S)
	Outcome			
	Survived	Died		
	Mean ± SD	Mean ± SD	t	p value
APACHE II Score	9.8 ± 6.8	21.6 ± 4.3	-6.74	<0.001 (S)

SD= standard deviation t: student's t test

P: < 0.05 significant (S)

Table (7): Pearson correlation between NSE and NFL Protein levels with APACHE II score, Total atropine dose and Hospital stay duration

		APACHE II score	Total atropine dose (mg)	Hospital stay duration (days)
NSE (ng/ml)	r	0.701	0.505	0.508
	p value	0.001 **(S)**	0.001 **(S)**	<0.001**(S)**
NFL (ng/ml)	r	0.438	0.468	0.305
	p value	0.001 **(S)**	0.001 **(S)**	0.031**(S)**

r: coefficient of correlation

P: < 0.05 significant (S) P: > 0.05 non significant (NS)

Table (8): ROC curve analysis to predict M.V. need

	Cutoff point	AUC	95% CI	p value	sig.	Sensitivity	Specificity	PPV	NPV
NSE (ng/ml)	>22.5	0.908	0.792 to 0.971	0.0001	S	81.82	94.12	96.4	72.7
NFL (ng/ml)	>16.5	0.799	0.662 to 0.899	0.0001	S	81.82	70.59	84.4	66.7

AUC: Area under Curve CI: confidence interval

PPV: Positive Predictive Value NPV: Negative Predictive Value

Table (9): ROC curve analysis to predict mortality

	Cutoff point	AUC	95% CI	p value	sig.	Sensitivity	Specificity	PPV	NPV
NSE (ng/ml)	>25	0.894	0.774 to 0.963	0.0001	S	94.74	74.19	69.2	95.8
NFL (ng/ml)	>19.8	0.693	0.546 to 0.815	0.0141	S	78.95	61.29	55.6	82.6

AUC: Area under Curve CI: confidence interval

PPV: Positive Predictive Value *NPV: Negative Predictive Value*

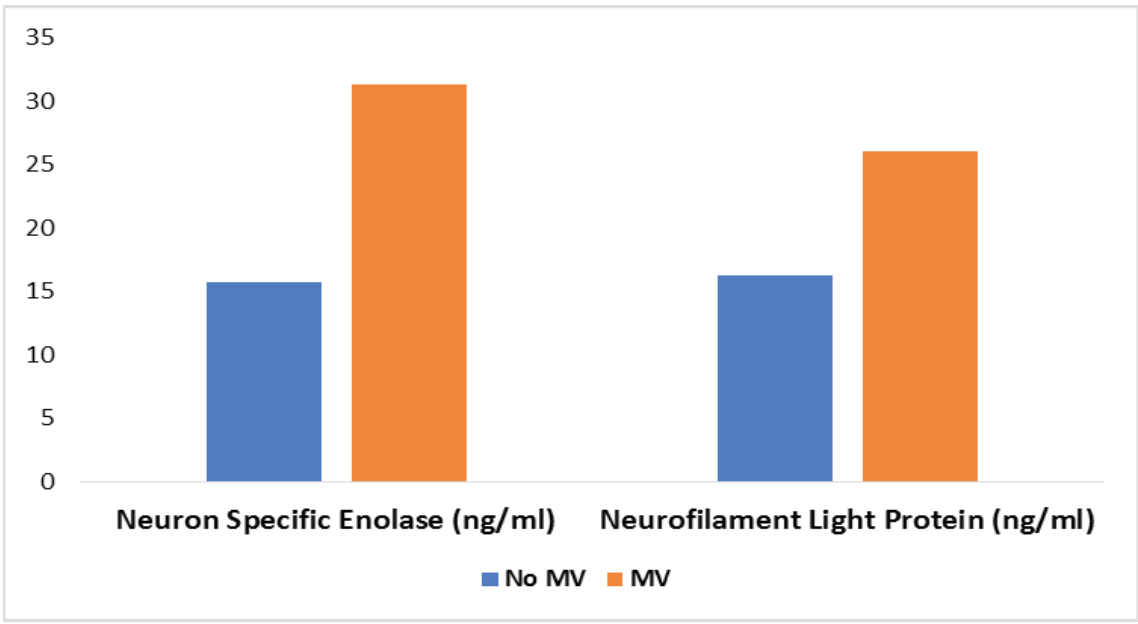

Figure (1): Differences in NSE and NFL protein levels in relation to M.V. need

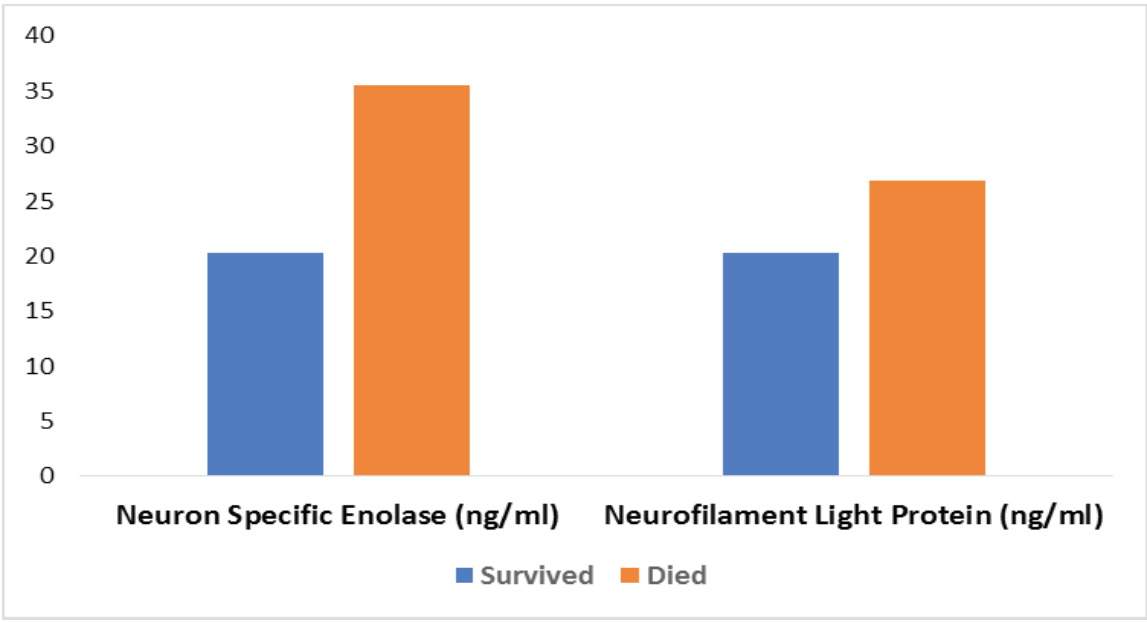

Figure (2): Differences in NSE and NFL protein levels in relation to outcome

Discussion

Despite the great developments in the ICU management, ingestion of OP compounds is still a greatly contributing agent of poisoning with high mortality rates, so it is important to estimate the severity and prognosis of the patients were intoxicated by these agents [7].

The study showed that the mean age of patients included was 32.3±12.6 years, this result was in agreement with the studies conducted by ***Kumar and Sahna (2017)*** [8] and ***Banday et al., (2015)*** [9] as it was noticed that the highest incidence of poisoning with OP insecticides was seen in the middle age group. That could be explained by increased stressful conditions as a result of unemployment, poverty and depression that concurrent with phase of life in which the people are

most ambitious and productive.

The study showed that 52% of cases were females and 48% were males, with female to male ratio was 1.08:1. This result was agreed with studies done by ***Hassan and Madboly (2013)*** [10] and ***Gündüz et al., (2015)*** [11] which showed that females were affected more than males with possible explanation of female predominance by the emotional liability of females to life stresses.

The study showed that 54% of cases were from rural areas and 46% were from urban areas which was in agreement with results of ***Bilal et al., (2014)*** [12] ***and Priyadarsini et al., (2015)*** [13]. This can be attributed to use of these compounds for agricultural as well as household purposes, so their easy availability is a major source of intoxication in rural areas.

In our study the suicidal exposure to OPCs was responsible for 78% of cases and accidental exposure was responsible for the remaining 22% of cases. These results agreed with ***Ismael et al., (2013)*** [14]***, Banday et al., (2015)*** [9]. Currently self-poisoning with pesticide has become a major clinical problem of the developing countries. These substances are readily available in shops and act as a common agent for suicidal purpose after trivial family problems [15].

The current study showed that the oral route was the main route of OP exposure; this was in accordance with the results of ***Bilal et al., (2014)*** [12]. Worldwide, the main route of intoxication recorded was the oral route due to easy oral intake of the poison especially that of liquid form as OP compounds. In addition, the lack of experience in dealing with these products by using protective equipment plays a significant role in exposure to these compounds through dermal and inhalation routes [16].

Most of patients included in the study were disturbed conscious level according to Reed's classification while only 12% of them were conscious. These results were in contrast to ***Girish et al., (2016)*** [17] results that recorded disturbance of conscious level in 22% of cases in their study, as well as ***Banday et al., (2015)*** [9] results that found 33% of the cases in their study with disturbed conscious level, the explanation of this variation may be attributed to that our study included only ICU admitted patients which already have more toxicity and more disturbance of consciousness than others admitted in ward.

We found that there was a significant correlation between APACHE II score and need to mechanical ventilation as APACHE II score was significantly high in mechanically ventilated patients than non mechanically ventilated .These results go with those of ***Kang et al., (2009)*** [18] who reported that the APACHE II score was high in patients who had complications especially respiratory failure.

We found that there was a significant difference between APACHE II score in survived and died patients and there was a significant correlation between APACHE II score and mortality. These results were similar to results conducted by ***Moussa et al., (2018)*** [19], ***Sumathi et al., (2014)*** [20] and ***Kim et al., (2013)*** [21].

The neurological effects of OP pesticides may be explained by the fact that OP compounds can inhibit cholinesterase enzyme, and resulting in accumulation of acetylcholine, which evokes the muscarinic and nicotinic receptors causing increasing in free radicals generation and increase in the oxidative stress. This process exhausted the nerve cells and may lead to degenerative effect to the nerves [22], [23].

Many neurochemical and immunohistological studies have confirmed that some isoenzymes or isoproteins, e.g. Neuron Specific Enolase (NSE) and Neurofilament Light (NFL) proteins are specifically distributed in neurons (NSE) and axons (NFL). Various clinical investigations have demonstrated the feasibility of using these protein markers for evaluating the pathological changes in the nervous system [24], [25].

Neuron Specific Enolase (NSE) level was initially analyzed in serum and ventricular CSF obtained from patients with severe head trauma, in whom it was identified as a promising marker of neuronal damage [26]. The study done by ***Borg et al. (2012)*** [27] found that NSE levels were significantly higher in the sera of TBI subjects than in control subjects.

NSE level in the present study revealed significant difference between cases and controls also there was a significant correlation between NSE levels and severity

of toxicity according to APACHE II score. These results are in a good agreement with ***Khater et al., (2015)*** [28] study who found that levels of NSE were significantly high in all groups compared to control group with positive correlation with severity. Also it was in accordance with results of ***Bozkurt et al. (2010)*** [24] who found that serum NSE level was markedly increased 2 hours after chlorpyrifos administration and remained high for 12 hours.

Our study showed that NSE had the higher sensitivity and specificity in predicting the need to M.V. and mortality, which was in agreement with ***Khater et al., (2015)*** [28] who found that NSE can be considered to be a good marker for predicting severity and outcome of acute OP poisoned patients.

However systematic studies which focus on peripheral nerve function with acute OP ingestion are scant, some previous studies had shed a lot of light on the neurotoxic consequences of acute high level pesticide exposure which may be associated with abnormalities in nerve function and increased risk of neurodegenerative diseases [29], [30].

Neurofilament light (NFL) protein is most abundant in the large-caliber myelinated axons that project into deeper brain layers and the spinal cord, ***Al Nimer et al., (2015)*** [31] in their study found an increase in serum NFL protein in patients with TBI, which in agreement with ***Shahim et al., (2016)*** [32] who found that serum NFL protein was markedly elevated in TBI patients compared to controls and suggests that measurement of serum NFL may be useful to assess the severity of neuronal injury following TBI.

Jayasinghe et al., (2012) [33] studied the effect of acute OP poisoning on peripheral nerves suggested that there may be sub-clinical sensory and motor neuropathies following single acute exposure to OP pesticides which may be due to distal demyelination process and axonal damage.

Our study showed that there was a significant difference in NFL protein level between cases and controls. There was a significant correlation between NFL protein level and severity according to APACHE II score, M.V. need and mortality.

These results agreed with results of ***McConnell et al. (1999)*** [34] study who Studied serum autoantibodies to neurofilament triplet proteins in a 16-year old boy after acute organophosphorus ingestion with predominance of anti NFL titers. The study conducted by ***Abou-Donia et al., (2018)*** [35] found elevated levels of autoantibodies to neurofilaments in patients with neurological symptoms and history of exposure to pesticides. Also the study conducted by ***El Rahman et al., (2018)*** [36] demonstrated the presence of serum autoantibodies to neurofilaments in a group of farmers chronically exposed to pesticides who developed neurological manifestations of neural injury, and these autoantibodies can be used as future diagnostic and/or therapeutic target for OP induced neurotoxicity.

Our study showed that there was a significant correlation between NSE and NFL protein levels with total atropine dose and hospital stay duration, as higher levels of these parameters associated with more sever toxicity and higher grades of APACHE II score with subsequent larger dose of atropine and longer stay duration. These results agreed with results of ***Kumar and Sahna (2017)*** [8] which found that patients with higher degrees of severity of poisoning required higher doses of atropine and also with ***Girish et al., (2016)*** [17] who observed significant association between the severity of poisoning and the increased hospitalization period.

Our study showed that 66% of cases were mechanically ventilated, these results were near to results of ***Kang et al. (2009)*** [18] which found that 51.5% of their cases were mechanically ventilated. In contrast, ***Kumar et al., (2017)*** [37] reported that 40% of their cases required mechanical ventilation, the explanation of this variation may be due to that our study included only ICU admitted patient which already in a high grade of toxicity with more possibility to respiratory failure and need to mechanical ventilation.

The study showed that 62% of cases were survived and 38% were died, this was greatly different from the results reported by ***Sun et al., (2015)*** [38] where deaths in their results were 12% of cases and recovery was observed in 88% of cases, also ***Bilal et al., (2014)*** [12] found that deaths represented 10% of their cases. The cause of this variation may be attributed to that our study included only ICU admitted patients who are already in

a higher degree of toxicity, in which a higher mortality rate is expected than ward admitted patients.

Conclusion

Neuron Specific Enolase (NSE) and Neurofilament Light (NFL) protein can reflect a degree of neurological affection of OP compounds and they were able to predict mechanical ventilation need and mortality in acute OP poisoned patients.

NSE and NFL protein levels have higher sensitivity and specificity in predicting morbidity and mortality, so they can be used as good markers for neurological damage associated with OP poisoning.

Conflict of Interest: The authors declare that there is no presence for conflict of interest.

Source of Fund: None

Ethical Consideration: The study was approved by the Fayoum faculty of medicine research ethical committee. An informed written consent was obtained from each patient or from his/her guardian for inclusion in the study and confidentiality was considered during this work.

References

1. Banerjee I., Tripathi S. and Roy A.S. Efficacy of pralidoxime in organophosphorus poisoning. Journal of postgraduate medicine.2014; 60(1):27-30.
2. Sawamoto K., Loro E. and Krajcic P. Time course of neuromuscular junction failure after acute parathion poisoning. Academic Emergency Medicine Journal. 2014; 21:180.
3. Bird S.B., Krajacic P., Sawamoto K., Bunya N. and Loro E. Pharmacotherapy to protect the neuromuscular junction after acute organophosphorus pesticide poisoning.Annals of the New York Academy of Sciences. 2016; 1374: 86–93.
4. El-Maraghi S., Yehia H., Hossam H., Yehia A. and Mowafy H. The prognostic value of neuron specific enolase in head injury. Egy J Crit Care Med. 2013; 1: 25–32.
5. Kuhle J., Plattner K., Bestwick J.P., et al. A comparative study of CSF neurofilament light and heavy chain protein in MS. Mult Scler. 2013; 19:1597–603.
6. Knaus W.A., Draper.E. A, Wagner D.P. and Zimmerman J.E. Apache II: a severity of disease classification system. Crit Care Med. 1985; 13: 818-829
7. Lee J., Lee Y., Park Y., et al. The difference in C-reactive protein value between initial and 24 hours follow-up (D-CRP) data as a predictor of mortality in organophosphate poisoned patients, Clinical Toxicology. 2013 51: 29–34
8. Kumar R. and Sahna E. Correlation of serum Pseudocholinesterase level and Peradeniya organophosphorus poisoning scale with the severity and in hospital outcome of acute organophosphorus poisoning. Inter J Contemporary Med Res. 2017; 4(8):1702-5.
9. Banday T., Tathineni B., Desai M., et al. Predictors of morbidity and mortality in organophosphorus poisoning: A case study in rural hospital in Karnataka, India, North Am J med Sci. 2015; 7(6): 259-65.
10. Hassan N. and Madboly A. Correlation between serum creatine phosphokinase and severity of acute organophosphorus poisoning: A Prospective clinical study. J Environ Sci, Toxicol and Food Technology. 2013; 4(5): 18-29.
11. Gündüz E, Dursun R, Icer M, et al. Factors affecting mortality in patients with organophosphate poisoning. J Pakistan Med Association (JPMA). 2015; 65(9): 967-72.
12. Bilal M., Khan Y., Ali S., et al. The pattern of organophosphorus poisoning and its short- term outcomes in various socioeconomic groups, Kaohsiung J Med Sci. 2014; 7(1): 11-6.
13. Priyadarsini C., Rao B. and Sarma M. Assessment of severity, treatment and outcome of organophosphorus poisoning: a descriptive study, J Evidence Based Medicine & Health Care. 2015; 2(21): 3194- 204.
14. Ismael M., Sherif M., El-Dahab F., et al. Cases with toxicity of anticholinestrase enzyme and factors affecting outcome. J Pharmacol and Toxicol. 2013; 8 (3): 90-7.
15. Ahasan H., Faruk A., Bala C., et al. Predicting the Short term Outcome in Acute Organophosphorus Compound (OPC) Poisoning with Poison Severity Scale on Hospital Admission in Dhaka Medical College Hospital. J Toxicol Cur Res. 2017; 1: 21-5.

16. Coskun R., Gundogan K., Sezgin G. et al. A retrospective review of intensive care management of organophosphate insecticide poisoning: Single center experience, Nigerian Journal of Clinical Practice. 2015; 18(5): 644-650.
17. Girish S., Kalyan and Reddy Y. To Assess the Severity of Organophosphorus Compound Poisoning Clinically by Using Peradeniya Score. Ind J Appl Res. 2016; 6 (4):617-9.
18. Kang E., Seok S., Lee K. et al. Factors for determining survival in acute organophosphate poisoning, Korean J Intern Med. 2009; 24: 362-367.
19. Moussa E.M., Mohamed A.S., Hilal A.M., Hasb Elnabi A.M. and Zaki A.N. The Role of APACHE II, SOFA, Serum amylase and Lipase in Assessment of Severity and Outcome of Acute Organophosphorus Poisoning. Ain Shams Journal of Forensic Medicine and Clinical Toxicology. 2018; 31: 41-50.
20. Sumathi M., Kumar S., Shashidhar K. et al. Prognostic significance of various biochemical parameters in acute organophosphorus poisoning. Toxicol Inter. 2014; 21:167-71.
21. Kim Y., Yeo J. and Kang M. et al. performance assessment of the SOFA, APACHE II scoring system, and SAPS II in Intensive Care Unit Organophosphate poisoned patients. J Korean Med Sci. 2013; 28:1822-1826.
22. Abou-Donia M.B. Organophosphorus ester induced chronic neurotoxicity. Arch Env Health. 2003; 58(8): 484-97.
23. Sen R., Nayak J. and Khadanga S. (2014): Study of serum cholinesterase, CPK and LDH as prognostic biomarkers in Organophosphorus Poisoning. Int J Med Res Rev. 2014; 2(3): 185 – 9.
24. Bozkurt A., Yardan T., Ciftcioglu E., et al. Time Course of Serum S100B Protein and Neuron-Specific Enolase Levels of a Single Dose of Chlorpyrifos in Rat. Basic Clini Pharmacol Toxicol. 2010; 107: 893–8.
25. Zetterberg H., Smith D.H. and Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. Nat Rev Neurol. 2013; 9:201-10.
26. Bohmer A. E., Oses J.P., Schmidt A.P. et al. (2011): Neuron-specific enolase, S100B, and glial fibrillary acidic protein levels as outcome predictors in patients with severe traumatic brain injury. Neurosurgery. 2011; 68:1624–1631.
27. Borg K., Bonomo J., Jauch E.C., Kupchak P., Stanton E.B., et al. Serum levels of biochemical markers of traumatic brain injury. ISRN Emerg Med. 2012; 1 –7.
28. Khater A.S., Medhat A.M., Mostafa A.O., Elshibiny H.A. and Abdel-Aziz M.A. Role of S100 beta protein, Neuron Specific Enolase enzyme and Myeline Basic Protein in acute organophosphorus insecticide poisoned patients. Egypt J. forensic Sci. appli. Toxicol. 2015; 15(1): 51-67.
29. Keifer M. and Mahurin R. Chronic neurologic effects of pesticide overexposure. Occup Med. 1997; 12:291–304.
30. Le Couteur D.G., McLean A.J., Taylor M.C., Woodham B.L. and Board P.G. Pesticides and Parkinson's disease. Biomed Pharmacother. 1999; 53:122–30.
31. Al Nimer F., Thelin E., Nyström H., Dring A.M., Svenningsson A., et al. Comparative Assessment of the Prognostic Value of Biomarkers in Traumatic Brain Injury Reveals an Independent Role for Serum Levels of Neurofilament Light. PLoS ONE. 2015; 10(7): e0132177.
32. Shahim P., Gren M., Liman V., Andreasson U. and Norgren N. et al. Serum neurofilament light protein predicts clinical outcome in traumatic brain injury. Sci Rep. 2016;6:36791.
33. Jayasinghe S.S., Pathirana K.D., Buckley N.A. Effects of Acute Organophosphorus Poisoning on Function of Peripheral Nerves: A Cohort Study. PLoS ONE. 2012; 7(11): e49405.
34. McConnell R., Delgado-Tellez E., Cuadra R. et al. Organophosphate neuropathy due to metamidophos: biochemical and neurophysiologic markers, Arch. Toxicol. 1999; 73: 296–300.
35. Abou-Donia M.B., Lieberman A. and Curtis L. Neural autoantibodies in patients with neurological symptoms and histories of chemical/mold exposures. Toxicology and Industrial Health. 2018; 34(1):44-53.
36. El Rahman H.A.A., Salama M., Gad El-Hak S.A. et al. A Panel of Autoantibodies Against Neural Proteins as Peripheral Biomarker for Pesticide-Induced Neurotoxicity. Neurotox Res. 2018; 33: 316–336.

37. Kumar R., Rao R. and Nrushen P. A study on serum cholinesterase levels as a prognostic marker in organophosphorus poisoning. Asia Pac J Health Sci. 2017; 4(1):91-9.

38. Sun I., Yoon H., and Lee K. Prognostic factors in cholinesterase inhibitor poisoning, Med Sci Monit. 2015; 21: 2900–2904