Original Article

A Retrospective Analysis of Serial Measurement of Serum Cholinesterase in Acute Poisoning with Organophosphate Compounds

M. S. Manu, Vishwanath Prashant, P. Akila, M. N. Suma, Hathur Basavanagowdappa[1]

Department of Biochemistry and [1]Medicine, JSS Medical College, JSS University, Mysore, India

ABSTRACT

Objectives: Retrospective analysis of the utility of serial measurements of serum acetylcholinesterase (AChE) in predicting the duration of stay in the intensive care unit (ICU), duration of mechanical ventilation (MV) and outcome of the patient from MV in organophosphate (OP) compound poisoning patients. **Materials and Methods:** The medical records of patients who presented to tertiary care hospital with symptomatic insecticidal poisoning from January 2009 to December 2010 were utilized for the study purpose. Forty four patients with history of poisoning were admitted during this period. Out of these 37 patients with history of OP poisoning, without any underlying diseases and whose serial AChE activity levels were available were included in the study. Data regarding clinical manifestation at presentation, AChE activity results and its interpretation, details of patient management and data on outcomes of patients were noted. **Results:** Serum acetycholinesterase levels below 1,250IU/L, 1,789IU/L and 2,764IU/L on day three, day four and five respectively indicates longer duration of stay in the ICU. Patients with serum AChE levels below 975IU/L, 876IU/L, 1,245IU/L, 1,395IU/L and 1,875IU/L on day one, two, three, four and five respectively take a longer time to be out of mechanical ventilation. Levels below 870IU/L, 1,110IU/L, 1,020IU/L and 885IU/L on day two, three, four and five respectively indicate poor prognosis of the patient and mortality. **Conclusion:** We conclude that the serial measurements of serum acetylcholinesterase levels can be useful in predicting the length of ICU stay, duration of mechanical ventilation and the prognosis of the patient with OP poisoning.

Key words: Mechanical ventilation, organophosphate compound, retrospective analysis, serum acetylcholinesterase levels

INTRODUCTION

Pesticides play an important role in the high productivity achieved in agriculture through the control of pests. However, pesticides are intentionally toxic, often towards non-target organisms. Organophosphate (OP) and carbamate pesticides are designed to inhibit acetylcholinesterase (AChE) and this enzyme has been used the most in enzymatic detection of these pesticides. Many enzymes used for the detection of pesticides are inhibited by the pesticide and the extent of inhibition is correlated to the concentration of the analyte. Other enzymatic methods such as the organophosphorus hydrolase assay use the analyte as a substrate, with the result that a positive signal is generated through the production of hydrolysis products rather than merely the inhibition of the enzyme. Evtugyn *et al*. gives a good overview on different enzymes that can be used for detection of toxicants.[1]

AChE catalyzes the hydrolysis of acetylcholine, which is a neurotransmitter[2] in the synaptic membrane to prevent its

Access this article online

Quick Response Code:

Website: www.toxicologyinternational.com

DOI: 10.4103/0971-6580.103662

Address for correspondence: Dr. Vishwanath Prashant, Associate Professor, Department of Biochemistry, JSS Medical College, JSS University, Mysore-570015, India. E-mail: drmvps@gmail.com

accumulation. This degradation process results in a lowered level of acetylcholine, and ultimately the termination of nerve impulses. OP compounds covalently block the active site of serine residue of AChE by undergoing nucleophilic attack to produce a serine-phosphoester adduct. This irreversible inactivation leads to an excess accumulation of acetylcholines in the peripheral and central nervous system causing cholinergic manifestations. At high doses, there is depression of the respiratory centre in the brain, followed by peripheral neuromuscular blocked causing respiratory paralysis and death.[3,4] AChE gene polymorphisms have been studied extensively in the recent past mainly due to the interest in treatment of Alzheimer's disease. However, AChE is a highly conserved molecule, and only a few naturally occurring genetic polymorphisms have been reported in the human gene.[5] Hence, in this study we planned to retrospectively analyze the utility of serial measurements of serum AChE in predicting the duration of stay in the ICU, duration of mechanical ventilation (MV) and outcome of the patient from MV in OP compound poisoning patients.

MATERIALS AND METHODS

The medical records of patients who presented to tertiary care hospital with symptomatic insecticidal poisoning from January 2009 to December 2010 were utilized for the study purpose. As a protocol in our study, all cases that presented with symptomatic organophosphate poisoning, whose serial AChE activity levels were available and who did not have any other underlying diseases were included in the study. Patients with history of other co-morbid conditions and chronic illness like diabetes mellitus, hypertension were excluded from the study. Data regarding clinical manifestation at presentation, AChE activity results and its interpretation, details of patient management, duration of stay in the ICU, duration of MV and data on outcomes of patients were noted. The measurement of AChE activity was conducted as described by others[6]. The procedure included addition of serum sample (20 µl) and 0.1 ml of 5% solution of acetylthiocholine iodide to 3 ml of dithiobisnitrobenzoic acid solution at 25°C. The mixture was mixed and the absorbance at 405 nm at 30 s interval was recorded for 2 min. The difference in absorbance was considered for estimation of AChE activity.

Statistical analysis was performed using the tabulated data collected from case records of patients with OP poisoning. One way ANOVA was used to estimate the utility of serial measurements of serum AChE. Receiver Operator Characteristics were constructed to determine the cut off levels of acetylcholinesterse to determine the length of stay in ICU, number of days on mechanical ventilation and the outcome of the patient. Differences were considered significant if the *P* value was <0.05. All statistical analysis was performed using MEDCALC statistical analysis software version 10.2.

RESULTS

Forty four patients with history of poisoning were admitted between January 2009 and December 2010 with an age range of 02 - 60 years. All of them were adults except a two year old girl where mother had consumed poison and fed it to the baby also. Majority of the cases were males (83.8%). 48.6% of the patients were smokers and 45.9% of the patients were alcoholics. Table 1 shows the poison compound used by the study group. Only patients with consumption of organophosphate compound were included in the study group.

The patients were divided into two groups based on the number of days they stayed in ICU; Group 1 < 7 days and Group 2 > 7 days. The POP score was significantly increased in group 2 (*P* value < 0.05) and the serum AChE levels on day three, four and five was significantly decreased in group 2 (*P* value < 0.05) [Table 2]. The ROC curves when constructed for the two groups did not show any significant cut off points for serum AChE levels to indicate the length of ICU stay on day 1 and day 2. However, ROC curves showed significant cut off points at 1,250 IU/L (sensitivity of 75 and specificity of 71.43), 1,789 IU/L (sensitivity of 81.25 and specificity of 76.19) and 2,764 IU/L (sensitivity of 87.5 and specificity of 66.67) on day three, four and five respectively [Figure 1].

Table 1: Distribution of subjects based on the substance used for poisoning

Poisoning compound	Frequency	Percentage
Organophosphate	37	84.1
Organochlorine	1	2.3
Carbamate	4	9.1
Unknown	2	4.5
Total	44	100.0

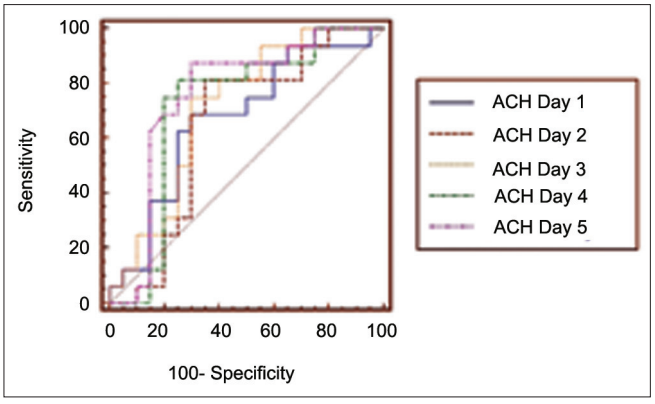

Figure 1: ROC curves of the acetylcholinesterase levels in the two groups of patients based on the number of days in the ICU

The patients were divided into three groups based on the number of days on mechanical ventilation; Group 1 not on MV, Group 2 on MV for <7 days and Group 3 on MV for >7 days. Younger patients were weaned from MV much earlier than the older patients. The POP score was significantly increased in group 2 when compared to group 1 (*P value* < 0.001) and in group 3 when compared to group 2 and group 1 (*P value* < 0.001). The serum AChE levels on day three and five were significantly decreased in group 3 when compared to group 1 (*P value* < 0.05) [Table 3]. The ROC curves when constructed for the two groups (MV <7 days and MV >7 days) showed significant cut off points at 975 IU/L (sensitivity of 80 and specificity of 68.2), 876 IU/L (sensitivity of 90 and specificity of 66.7), 1,245 IU/L (sensitivity of 90 and specificity of 68.2), 1,395 IU/L (sensitivity of 90 and specificity of 72.7) and 1,875 IU/L (sensitivity of 80 and specificity of 81.8) on day one, two, three, four and five respectively [Figure 2].

The patients were divided into two groups based on the condition of the patient at the time of discharge; Group 1 survivors and Group 2 non survivors. None of the variables showed significant changes among the two groups [Table 4]. The ROC curves when constructed for the two groups did not show any significant cut off points for serum AChE levels to indicate condition of the patient at the time of discharge on day one. However, ROC curves showed significant cut off points at 870 IU/L (sensitivity of 68.7 and specificity of 100), 1,110 IU/L (sensitivity of

Table 2: Variables in the two groups based on the number of days in ICU

Variables	No. of days in ICU			
	<7 days (*n* = 21)		>7 days (*n* = 16)	
	Mean	SD	Mean	SD
Age (Years)	28.143	11.3767	33.688	10.9223
Poisoning severity (POP Scale)	6.333	1.6228	8.125*	1.2583
AChE Day1 (IU/L)	2985.476	3041.9829	1507.813	1788.3699
AChE Day2 (IU/L)	2866.100	3347.2312	1281.625	1254.3477
AChE Day3 (IU/L)	3589.286	3564.1898	1414.125*	1168.4182
AChE Day4 (IU/L)	4116.190	3838.0330	1775.563*	1688.1269
AChE Day5 (IU/L)	4755.095	4004.9797	2123.875*	1778.0634

* *P* value < 0.05, n = Number of patients, SD = Standard deviation, AChE = acetylcholinesterase

Table 3: Variables in the three groups based on the number of days on mechanical ventilator

Variables	No. of days on mechanical ventilation					
	Not on MV (*n* = 5)		On MV <7 days (*n* = 22)		On MV >7 days (*n* = 10)	
	Mean	SD	Mean	SD	Mean	SD
Age (Years)	21.400	13.4833	29.318	9.3370	37.800*	11.1634
Poisoning severity (POP Scale)	5.000	1.0000	6.909	1.5090	8.600#	0.9661
AChE Day1 (IU/L)	3896.800	2752.8988	2585.545	2983.4939	1045.400	776.9591
AChE Day2 (IU/L)	4257.600	2711.9578	2243.905	3061.9409	941.800	736.0665
AChE Day3 (IU/L)	4751.800	1721.2390	2907.091	3464.6676	1028.600*	645.3504
AChE Day4 (IU/L)	5387.000	1788.6570	3368.364	3764.6269	1381.000	1468.3481
AChE Day5 (IU/L)	6597.200	1517.4695	3829.045	3889.4430	1661.400*	1511.3017

**P* value < 0.05, #P value < 0.001, n = Number of patients, AChE = acetylcholinesterase, SD = Standard deviation

Table 4: Variables in the two groups based on the condition at the time of discharge

Variables	Condition at the time of discharge			
	Survivors (*n* = 33)		Non-survivors (*n* = 4)	
	Mean	SD	Mean	SD
Age (Years)	30.273	10.9467	32.750	16.3376
Poisoning severity (POP Scale)	6.970	1.7227	8.250	1.2583
AChE Day1 (IU/L)	2528.212	2753.5165	847.250	242.0157
AChE Day2 (IU/L)	2346.625	2833.3577	684.000	180.7392
AChE Day3 (IU/L)	2874.485	3069.8219	785.750	358.7463
AChE Day4 (IU/L)	3407.576	3348.1226	599.750	296.4775
AChE Day5 (IU/L)	3988.212	3486.0502	557.000	263.2451

n = Number of patients, SD = Standard deviation, AChE = acetylcholinesterase

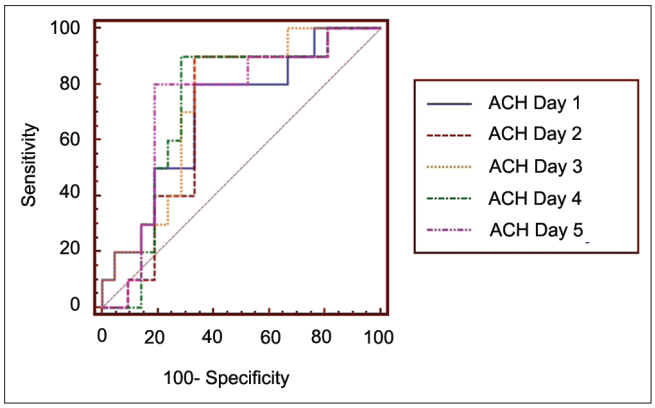

Figure 2: ROC curves of the acetylcholinesterase levels in the two groups of patients based on the number of days on mechanical ventilation

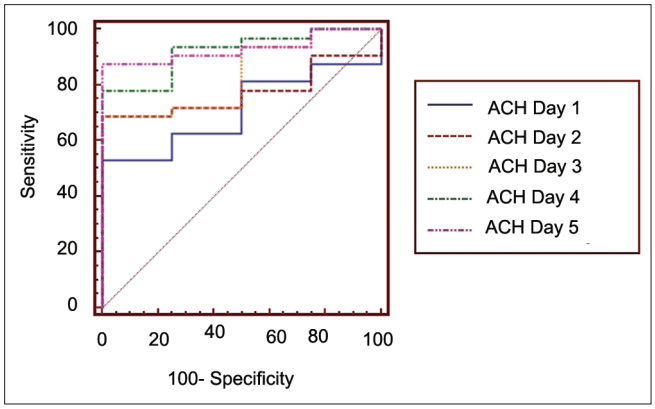

Figure 3: ROC curves of the AChE levels in the two groups based on the condition of the patient at the time of discharge

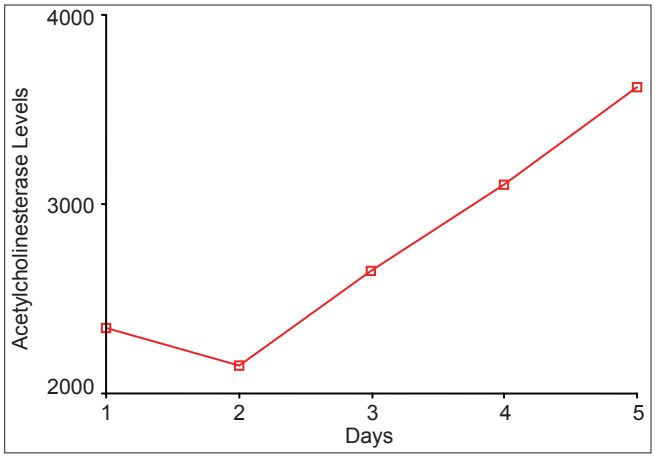

Figure 4: Mean serial AChE levels

61.7 and specificity of 100), 1,020 IU/L (sensitivity of 78.8 and specificity of 100) and 885 IU/L (sensitivity of 87.9 and specificity of 100) on day 2 (*P value* < 0.05), day three (*P value* < 0.0001), day 4 (*P value* < 0.0001) and day 5 (*P value* < 0.0001) respectively [Figure 3].

Table 5: Average number of days in the hospital, ICU and on mechanical ventilation

	Mean	SD	<7 days *n* (%)	>7 days *n* (%)
No. days in hospital	11.541	5.4650	9 (24.3)	28 (75.7)
No. of days in intensive care unit	7.324	3.9934	21 (56.8)	16 (43.2)
No. of days on mechanical ventilation	5.081	4.2386	22 (68.8)	10 (31.2)

DISCUSSION

The majority of poisoning cases were due to intentional oral ingestion of the insecticide (attempted suicide). The poisoning severity at the time of admission was determined based on Peradeniya organophosphate poisoning scale (POP scale).[7] However, all the patients included in the study belonged to the moderate poisoning category as per the POP scale. The average number of days stayed by the patients in the hospital, ICU and on MV is shown in Table 5. Table 5 also shows the number of patients who stayed in the hospital, ICU and on MV for <7 days and >7 days. Among the 37 patients only nine patients were discharged from the hospital within seven days. All of them were admitted in the ICU and 32 of them were intubated and put on MV.

The mean serum AChE levels were 2,346.5 IU/L ±2,650.3, 2,161.8 IU/L ±2,719.2, 2,648.6 IU/L ±2,969.8, 3,104 IU/L ±3,279.1, 3,617.2 IU/L ±3,460.4 on day one, two, three, four and five respectively [Figure 4]. Two mg of atropine was given as a test dose followed by 2 mg every 10–20 min as required to keep the patient atropinized. A bolus dose of oxime was instituted in 7 patients. Thirty three patients improved and were discharged from the hospital however; four patients succumbed to the poisonous compound.

With the increase in the use of OP compounds for agricultural and industrial purposes and due to easy access and low cost, OP poisoning is becoming the commonest cause of morbidity and mortality. All signs and symptoms of OP poisoning are cholinergic in nature and affect muscarinic, nicotinic and central nervous system receptors.[8] The critical symptoms in the management are respiratory symptoms. Depression of plasma pseudocholinesterase and/or RBC acetylcholinesterase enzyme activities are generally available biochemical indicators for excessive absorption of OP compound.

The enzyme depression is usually apparent within a few minutes to hours after significant consumption of OP compounds. Paraoxonases (PON) are a group of enzymes involved in the hydrolysis of organophosphates. The three known isoforms of PON (PON1, PON2, PON3) are coded by a PON set of genes on located on the long arm of chromosome 7. Reduced PON1 activities could be

expected to subject carriers of this *ACHE* polymorphism to additional risk because of the insufficient PON1 capacity to hydrolyze organophosphate (OP) AChE inhibitors. Constitutive AChE overproduction such as occurs in carriers of the Δ*ACHE* mutation prevents sufficient overproduction of the scavenging AChE molecules upon exposure to anti-AChEs.[9]

The standard treatment consists of reversal of the biochemical effects of acetylcholine with atropine for either OPs or carbamates poisoning and reactivation of the inhibited acetylcholinesterase with an oxime antidote, which should be administered as early as possible for OPs but it is contraindicated in carbamates poisoning.[10] Studies have shown that differential diagnosis of either carbamates or OPs poisoning can be achieved and the efficacy of antidote therapy can be evaluated by utilizing serial measurements of BuChE activities and the proposed model curves.[11] However, the cut off levels of the enzymes below which the patient will have poor prognosis has not yet been determined. Hence, in this study we have attempted to document the critical levels of the enzymes from day one to five.

Serum acetycholinesterase levels below 1,250 IU/L, 1,789 IU/L and 2,764 IU/L on day three, four and five respectively indicates longer duration of stay in the ICU. Patients with serum acetylcholinesterase levels below 975 IU/L, 876 IU/L, 1,245 IU/L, 1,395 IU/L and 1,875 IU/L on day one, two, three, four and five respectively take a longer time to be out of mechanical ventilation. Levels below 870 IU/L, 1,110 IU/L, 1,020 IU/L and 885 IU/L on day two, three, four and five respectively indicate poor prognosis of the patient and mortality.

However, small sample size is the limitation of the study. The data of this retrospective study can be taken as a preliminary data for the conduct of large scale prospective study and validate these findings. We conclude that the serial measurements of serum acetylcholinesterase can be useful in predicting the length of ICU stay, duration of mechanical ventilation and the prognosis of the patient with OP poisoning.

REFERENCES

1. Evtugyn GA, Budnikov HC, Nikolskaya EB. Sensitivity and selectivity of electrochemical enzyme sensors for inhibitor determination. Talanta 1998;46:465-84.
2. Fukuto TR. Mechanism of action of organophosphorus and carbamate insecticides. Environ Health Perspect 1990;87:245-54.
3. Bajgar J. Organophosphates/nerve agent poisoning: Mechanism of action, diagnosis, prophylaxis, and treatment. Adv Clin Chem 2004;38:151-216.
4. Ganesan K, Raza SK, Vijayaraghavan R. Chemical warfare agents. J Pharm Bioallied Sci 2010;2:166-78.
5. Hasin Y, Avidan N, Bercovich D, Korczyn AD, Silman I, Beckmann JS, *et al*. Analysis of genetic polymorphisms in acetylcholinesterase as reflected in different populations. Curr Alzheimer Res 2005;2:207-18.
6. Dyer SM, Cattani M, Pisaniello DL, Williams FM, Edwards JW. Peripheral cholinesterase inhibition by occupational chlorpyrifos exposure in Australian termiticide applicators. Toxicology 2001;169:177-85.
7. Senanayake N, de Silva HJ, Karalliedde L. A scale to assess severity in organophosphorus intoxication: POP scale. Hum Exp Toxicol 1993;12:297-9.
8. Taylor P. Anticholinesterase agents. In: Gilman AG, Goodman LS, editors. The pharmacological basis of therapeutics. New York: Macmillan Publishing Co. Inc.; 1985. p. 110-28.
9. Benmoyal-Segal L, Vander T, Shifman S, Bryk B, Ebstein RP, Marcus EL, *et al*. Acetylcholinesterase/paraoxonase interactions increase the risk of insecticide-induced Parkinson's disease. FASEB J 2005;19:452-4.
10. Ellenhorn MJ, Pesticides, in: M.J. Ellenhorn, editor. Ellenhorn's Medical Toxicology Diagnosis and Treatment of Human Poisoning, second ed., Baltimore, USA: William and Wilkins; 1997, p. 1614-63.
11. Abdullat IM, Battah AH, Hadidi KA. The use of serial measurement of plasma cholinesterase in the management of acute poisoning with organophosphates and carbamates. Forensic Sci Int 2006;162:126-30.

How to cite this article: Manu MS, Prashant V, Akila P, Suma MN, Basavanagowdappa H. A retrospective analysis of serial measurement of serum cholinesterase in acute poisoning with organophosphate compounds. Toxicol Int 2012;19:255-9.

Source of Support: Nil. **Conflict of Interest:** None declared.