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Cases with Toxicity of Anticholinesterase Enzyme and Factors Affecting Outcome

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ABSTRACT

The present study was designed to investigate outcomes and predictors of mortality in cases of acute anticholinesterase insecticide poisoning. One hundred cases diagnosed to have acute Organophosphate (OP) toxicity and admitted to Poison Control Center (PCC) were included in the study. Two sheets, one before and the other 24 h after therapy were used for each patient. These sheets included (1) Sociodemographic, (2) Clinical and (3) Investigations; thereafter 5 mL of venous blood were collected on admission after the diagnosis but before any medications were given. Another sample was obtained 24 h later after medications. Serum was used for estimation of pseudocholine esterase level, liver and renal function tests, tests for acute pancreatitis, serum electrolytes, fasting and postprandial glucose levels. Intoxicated cases were mainly males, less than 20 years, residing in urban areas, cause was mainly suicide; delay time correlates with severity; cholinesterase levels negatively correlated with severity. There was powerful, inverse, significant correlation between cholinesterase levels and glucose, lipase, creatinine, Alanine Transaminase (ALT) and Aspartate Transaminase (AST). Morality rate was 7%; one in the moderate group and 6 cases in the severe group (24%). Serum cholinesterase, delay time, sodium, glucose, amylase, lipase, SGPT and alkaline phosphatase, measured on the first day can be used as predictors of mortality in studied cases. Delayed time is the powerful single predictor, followed by lipase and alkaline phosphatase. The delay time was the most useful single predictor of mortality in the present study.

Key words: Cholinesterase, predictors, toxicity

INTRODUCTION

Anticholinesterase insecticides are possibly the most widely used insecticides worldwide that may cause serious poisoning either through suicidal attempt or on accidental exposure. Toxicity is produced by acetylcholine accumulation at cholinergic receptors resulting in a variety of complications including respiratory failure, cardiovascular and central nervous system depression or seizures. Childhood toxicity is associated with predominance of central nervous system manifestations (Lifshitz *et al*., 1999).

Anticholinesterase insecticide poisoning represents a major health problem worldwide, as it is associated with high morbidity and mortality. Worldwide studies report mortality rates from 3% to 30%; that increased to 50% in ventilated patients (Sungur and Guven, 2001). Furthermore,

animal LD_{50} can not be used to estimate the severity of human toxicity and is probably of limited value in risk assessment or management of human poisoning (Eddleston *et al*., 2008). About 35% of patients acutely intoxicated with anticholinesterase insecticides require intensive care management and mechanical ventilation and despite proper medical intervention, mortality rate remains high and this necessitates the performance of further research on the other factors that may affect the final outcome (Munidasa *et al*., 2004). The main cause of death due to acute anticholinesterase insecticide poisoning is believed to be due to acute respiratory failure caused by the peripheral and central cholinergic actions of these agents (Asari *et al*., 2004). There are several studies reporting respiratory failure in 16, 17, 35, 68, 74, 75 and 78% of cases (ElMazoudy *et al*., 2011).

The aim of the present study is to study the outcomes and predictors of mortality of 100 cases of acute anticholinesterase insecticide poisoning requiring admission in an inpatient or intensive care therapy.

MATERIALS AND METHODS

Patients were recruited from those admitted to Poison Control Center (PCC), Faculty of Medicine, Ain Shams University from January 1st, 2011, onwards. Furthermore, only those patients who were admitted to an inpatients or intensive care units after diagnosis of having organophosphate poisoning were included in the study. Diagnosis of organophosphate toxicity was accomplished by: History of exposures, symptoms and signs of muscarinic and nicotinic simulation, confirmed by depressed pseudocholinesterase levels and response to an atropine oximes therapy. Exclusion criteria: Patients with history of renal, hepatic, pancreatic or cardiac diseases; and cases that responded to atropine only (carbamates) were excluded from the study.

One hundred patients were divided into 4 groups of 25 each according to the severity of toxicity. Severity of toxicity was decided by the level of enzyme pseudocholinesterase in the blood. Group 1: Included cases with normal choline esterase levels; Group 2: Included cases with pseudocholinesterase levels 20-50% of the normal value (mild group); Group 3: Included cases with pseudocholinesterase levels 10-20% of the normal values (Moderate group); Group 4: Included cases with pseudocholinesterase levels of less than 10% of normal values (Severe group). The studied parameters were tested in groups II, III and IV twice; on admission and 24 h later.

Two special observation sheets were created for each patient in this study. One before therapy and the other 24 h later. These sheets were designed to collect data into 3 main parts; (1) Sociodemographic study, (2) Clinical evaluation and (3) Investigations.

Sampling: Venous blood (5 mL) were collected from each patient on admission immediately after the diagnosis of poisoning but before any medication was given. Another sample was obtained from each patient 24 h later as a follow up measure and to pick up any probable delayed toxic effects. Controls were sampled only once on admission. Venous blood samples were kept in a clean dry centrifuge tubes and left to stand for few hours before centrifugation to avoid hemolysis.

Laboratory investigations: Pseudocholinesterase levels were determined according to the method of Osawa *et al*. (2005), liver function tests, renal function tests, tests for acute pancreatitis according to Carroll *et al*. (2007), serum electrolytes, fasting and postprandial glucose level.

Statistical analysis of data: The collected data were organized, tabulated and statistically analyzed using Statistical Package for Social Sciences (SPSS) version 16. For qualitative data,

frequency and percent distribution were calculated and for comparison between groups, the chi square test was used. For quantitative data, mean, standard deviation and range (minimum and maximum) were calculated and for comparison between groups, the one way analysis of variance was used. For comparison between two means, the students (t) test was used. For correlation between two parameters, the two ways spearman correlation coefficient (r) was used. For prediction of mortality, the simple linear regression was calculated. For interpretation of results, p value = 0.05 was considered significant.

RESULTS

The present study included 100 cases; 81% of them were males and 19% were females and there was statistically insignificant difference between studied groups as regard sex distribution (males represent 72, 76, 92.0 and 84% of control, mild, moderate and severe groups, respectively). In all cases, age ranged from 14 to 41 years with a mean of 22.52±6.27 years and there was a statistically insignificant difference between groups as regard age. Urban cases were 63 and 37% live in rural areas, with a statistically insignificant difference between groups as regards residence. Mode of toxicity was suicidal in 70% of cases, accidental in 22%, occupational in 6% and homicidal in 2% with a statistically insignificant difference between groups as regards mode of toxicity. Delay time ranged from 0.5 to 5.0 h with a mean of 2.62±0.92 h and there was statistically insignificant variance between different studied groups as regards time of delay (Table 1). In the present study, there was a statistically significant increase of cholinesterase in the second day in comparison to their values in the first day in mild (524.0±52.67 vs. 1013.6±210.49), moderate (351.56±53.99 vs. 944.20±62.79) and severe groups (140.44±18.23 vs. 793.80±45.53) (Table 2).

As regards morality rate, it was found to be 7%; 1 of them was in the moderate group representing 4% of this group and 6 cases in the severe group, representing 24% of this group and

Table 1: Personal characteristics, mode of toxicity and delay time in studied cases

Variables	Control g.	Mild g.	Moderate g.	Severe g.	Total	p-value
Male gender (n,%)	18(72.0%)	19(76.0%)	23(92.0%)	21(84.0%)	81(81.0%)	0.28 (NS)
Age (mean±SD)	22.28±7.21	23.12±6.67	23.24±6.66	21.44±4.39	22.52±6.27	0.72 (NS)
Residence (urban)	16(64.0%)	18(72.0%)	15(60.0%)	14(56.0%)	63(63.0%)	0.68 (NS)
Toxicity mode (n,%)						
Suicidal	19(76.0%)	18(72.0%)	17(68.0%)	16(64.0%)	70(70.0%)	
Accidental	4(16.0%)	5(20.0%)	7(28.0%)	6(24.0%)	22(22.0%)	0.92 (NS)
Occupational	2(8.0%)	1(4.0%)	1(4.0%)	2(8.0%)	6(6.0%)	
Homicidal	0(0.0%)	1(4.0%)	0(0.0%)	1(4.0%)	2(2.0%)	
Delay time	2.46±1.11	2.60±0.95	2.76±0.77	2.68±0.85	2.62±0.92	0.70 (NS)

(NB) NS.: Non-significant

Table 2: Pseudocholine- esterase levels in studied groups as the first and second day

	First day		Second day			
Parameters	Mean	SD	Mean	SD	Paired (t)	p-value
Control group	2781.6	242.76	2739.7	192.54	1.37	0.18 (NS)
Mild group	524.00	52.67	1013.6	210.49	10.84	<0.001 (S)
Moderate group	351.56	53.99	944.20	62.79	38.69	<0.001 (S)
Severe group	140.44	18.23	793.80	45.53	63.11	<0.001 (S)
Total	949.40	1079.20	1237.6	716.43	17.32	<0.001 (S)

(NB) NS.: Non-significant, S: Significant

there was a statistically significant difference between groups as regards mortality rate (Table 3). As regards clinical signs and symptoms, it must be said that, OP poisoning typically presented with one of the following categories: Muscarinic, nicotinic and CNS. In the present study, patients presented with a mixture of these manifestations (for details of clinical presentation revise tables in results). In dead cases, there was a statistically significant increase of cholinesterase, sodium, glucose, amylase, lipase, SGPT, alkaline phosphatase and delay time in comparison to living cases. As regards correlation between cholinesterase on the first day with different studied parameters, there was a powerful, proportional and statistically significant correlation between serum potassium and cholinesterase. On the other hand, there was powerful, inverse (negative), statistically significant correlation between cholinesterase levels and glucose, lipase, creatinine, SGOPT and SGPT. The correlation with blood urea was moderate, inverse and statistically significant. On the other hand, no correlation was found with sodium and total bilirubin levels (Data not presented).

Using simple linear regression, it was found that, serum cholinesterase, delay time ($\beta = 0.36$; $p<0.001$), sodium ($\beta = 0.32$; $p = 0.001$), glucose, amylase, lipase, SGPT and alkaline phosphatase, measured in the first day can be used as predictors of mortality in studied cases. Delay time is the powerful single predictor, followed by lipase and alkaline phosphatase while cholinesterase is the least one (Table 4).

Table 3: Comparison between different studied groups as regards mortality rate

	Mortality					
	Yes		No		Statistics	
Groups	n	%	n	%	χ^2	p-value
Control group	0	0.0	25	100.0	15.20	0.002(S)
Mild group	0	0.0	25	100.0		
Moderate group	1	4.0	24	96.0		
Severe group	6	24.0	19	76.0		
Total	7	7.0	93	93.0		

S: Significant

Table 4: Simple linear regression analysis of different parameters as predictors of mortality

Parameters	β	p-value
Cholinesterase on first day	0.190	0.05 (S)
Delay time	0.360	<0.001 (S)
Age	0.035	0.73 (NS)
Sodium	0.320	0.001 (S)
Potassium	0.089	0.38 (NS)
Glucose	0.410	0.042 (S)
Amylase	0.250	0.014 (S)
Lipase	0.260	0.006 (S)
Urea	-0.069	0.34 (NS)
Creatinine	-0.160	0.11 (NS)
SGOT	-0.190	0.053 (NS)
SGPT	-0.230	0.024 (S)
Alkaline phosphatase	-0.250	0.009 (S)
Total bilirubin	-0.083	0.41 (NS)

(NB) NS.: Non-significant, S: Significant

DISCUSSION

The incidence of pesticide toxicity has been doubled in developing countries during 1990s, as reported by the World Health Organization (WHO) (WHO, 2001). The present study was designed to follow up acute cases of anticholinesterase insecticides referred to Poison Control Center, of Ain Shams University; to study the outcome and predictors of mortality in patients with acute severe anticholinesterase insecticide poisoning.

In the present study, males were more affected than females; the most common affected age group was those below twenty years of age and the majority of cases were of urban areas. Suicidal attempts were the most common modes of toxicity followed by the accidental mode. These findings were in agreement with that of Saadeh (2001) who reported that a female to male ratio of 1: 1.1 and predominant poisoning in age group 15-19 years, but contradictory to results of the present study, come from rural areas. The majorities were due to commission of suicide, then accidental exposure (26.0%) and remaining 10% were from occupational exposure. Mortality rate was 4%.

As regard time of delay (time elapsed between poisoning and presentation at the emergency room in the PCC) ranged from 0.5 to 5.0 h with a mean of 2.62±0.92 h and there was a statistically insignificant variance between different studied groups as regards time of delay. Better prognosis and outcome was noted with those who arrived earliest and vice versa. These results are supported by those reported by Burns *et al.* (1998) who reported that death following OP ingestion can be a complication of delayed or inadequate treatment. This can be explained by the fact that, the more the delay, allows longer gastrointestinal absorption, hepatic conversion of OP agents to more toxic ones, irreversible inhibition of AChE "aging" and delayed respiratory management and subsequent hypoxic tissue damage.

As regards clinical signs and symptoms, it must be said that, OP poisoning typically presented with one of the following categories: muscarinic, nicotinic and CNS. In the present study, patients presented with a mixture of these manifestations (for details of clinical presentation revise tables in results). This is in agreement with Peter and Cherian (2000) who reported that, with OP insecticides; cases presented different clinical manifestations ranging from GIT manifestations as nausea, vomiting or diarrhea, to neurological symptoms, as seizures, muscle weekness or cardiac manifestations as conduction abnormalities. Saadeh (2001) reported that, muscarinc effects of OP insecticide toxicity were more prevalent followed by CNS or nicotinic manifestations.

As regards morality rate, it was found to be 7%; 1 of them was in the moderate group representing 4% of this group and 6 cases in the severe group, representing 24% of this group and there was statistically significant difference between groups as regards mortality rate. This rate of mortality is lower than that reported in previous reports, that found estimated mortality following OP ingestion to range from 20 to 50% (Yamanaka *et al.*, 1993; Yamashita *et al.*, 1997; Munidasa *et al.*, 2004). This can be explained by the availability of immediate resuscitation, including circulatory support and mechanical ventilation when indicated.

In dead cases, there was statistically significant increase of cholinesterase, increase of sodium, glucose, amylase, lipase, SGPT, alkaline phosphatase and delay time in comparison to living cases. Using simple linear regression, it was found that, serum cholinesterase, delay time, sodium, glucose, amylase, lipase, SGPT and alkaline phosphatase, measured on the first day can be used as predictors of mortality in studied cases. Delay time is the powerful single predictor, followed by lipase and alkaline phosphatase and cholinesterase is the least one. In their study, Kang *et al.* (2009) total mortality rate was 19% (13 deaths in 68 patients). Age, amount ingested, APACHE II score, basal cholinesterase levels and respiratory with mechanical ventilation were significantly associated with a poor outcome (Aygun *et al.*, 2002).

Using simple linear regression, it was found that, serum cholinesterase, delay time, sodium, glucose, amylase, lipase, SGPT and alkaline phosphatase, measured in the first day can be used as predictors of mortality in the studied cases. Delay time is the powerful single predictor, followed by lipase and alkaline phosphatase and cholinesterase is the least one. These results go in agreement with Kaur *et al.* (2007) who detected significant increase of SGOT and SGPT levels following treatment of experimental animals with chlorpyrifos in a single oral dose of 200 mg kg^{-1} b.wt. In addition, Akhtar *et al.* (2009) detected significant increase in serum concentrations of SGOT and SGPT in rats exposed to OP insecticides namely dichlorofos dimethoate.

Regarding the mechanism of OP-induced liver toxicity, Akhgari *et al.* (2003) reported that, the toxic effects of OP agents on the liver are mediated through AChE inhibition leading to vascular disturbance. This vascular disturbance results in vasogenic edema and hepatocellular damage. Furthermore, Buyukokuroglu *et al.* (2008) confirmed the evidence for the occurrence of OP-induced oxidative tissue damage which are DNA strand Breaks, increased activities of antioxidant enzymes and down-regulation of glutathione peroxidase activity and glutathione.

Weizman and Sofer (1992) demonstrated significant elevation of serum levels of amylase in 5 out of 17 subjects with typical OP poisoning. Moritz *et al.* (1994) reported increased levels of amylase and lipase in one female patient with anticholinesterase insecticide poisoning in a suicidal attempt. In addition, Verger *et al.* (1996) stated that; OP increase sensitivity of human exocrine pancreas to acetylcholine. The improvement of results on the second day with treatment supports these mechanisms.

Pope and Olson (1990) reported that dehydration from excessive sweating, hyperventilation, diarrhea and osmotic diuresis as from hyperglycemia may cause disproportional water loss and subsequent hypernatremia, even with normal kidney functions. Moritz *et al.* (1994) reported hypokalemia in one female patient with anticholinesterase insecticide poisoning after committing suicide. In addition, Osmundson (1998) reported that, hypokalemic together with hyperglycemia is a well documented finding in OP intoxication. Hypokalemia which add to muscle weakness and hyporeflexia may be caused by beta-2 agonist intoxication that leads to intracellular shift of potassium. This may explain hypokalemia as a result of sympathetic overactivity in OP toxicity.

Sharma (1999) studied the effects of single and repeated disease of monocrotoplios insecticide (2 mg kg^{-1}) on blood glucose levels of rats and reported hyperglycemia. Jyothi and Narayan (1999) noticed high levels of serum glucose in fresh water catfish exposed to sublethal concentration of phorate.

CONCLUSION

In short, the results of the present study, revealed the importance of different clinical parameters of organophosphate intoxication in diagnosis, follow up and prediction of mortality in acute organophosphate toxicity. The delay time was the most useful single predictor of mortality in the present study.

REFERENCES

Akhgari, M., M. Abdollahi, A. Kebryaeezadeh, R. Hosseini and O. Sabzevari, 2003. Biochemical evidence for free radicalinduced lipid peroxidation as a mechanism for Subchronic toxicity of malathion in blood and liver of rats. Hum. Exp. Toxicol., 22: 205-211.

Akhtar, N., M.K. Srivastava and R.B. Raizada, 2009. Assessment of chlorpyrifos toxicity on certain organs in rat, *Rattus norvegicus*. J. Environ. Biol., 30: 1047-1053.

Asari, Y., Y. Kamijyo and K. Soma, 2004. Changes in the hemodynamic state of patients with acute lethal organophosphate poisoning. Vet. Hum. Toxicol., 46: 5-9.

Aygun, D., Z. Doganay, L. Altintop, H. Guven, M. Onar, T. Deniz and T. Sunter, 2002. Serum acetylcholinesterase and prognosis of acute organophosphate poisoning. J. Toxicol. Clin. Toxicol., 40: 903-910.

Burns, C.J., J.B. Cartmill, B.S. Powers and M.K. Lee, 1998. Update of the morbidity experience of employees potentially exposed to chlorpyrifos. Occup. Environ. Med., 55: 65-70.

Buyukokuroglu, M.E., M. Cemek, Y. Yurumez, Y. Yavuz and A. Aslan, 2008. Antioxidative role of melatonin in organophosphate toxicity in rats. Cell Biol. Toxicol., 24: 151-158.

Carroll, J.K., B. Herrick, T. Gipson and S.P. Lee, 2007. Acute pancreatitis: Diagnosis, prognosis and treatment. Am. Family Physician, 75: 1513-1520.

Eddleston, M., N.A. Buckley, P. Eyer and A.H. Dawson, 2008. Management of acute organophosphorus pesticide poisoning. Lancet, 371: 597-607.

ElMazoudy, R.H., A.A. Attia and H.S. AbdElGawad, 2011. Evaluation of developmental toxicity induced by anticholinesterase insecticide, diazinon in female rats. Birth Defects Res. Part B: Dev. Reprod. Toxicol., 92: 534-542.

Jyothi, B. and G. Narayan, 1999. Certain pesticide-induced carbohydrate metabolic disorders in the serum of freshwater fish *Clarias batrachus* (Linn.). Food Chem. Toxicol., 37: 417-421.

Kang, E.J., S.J. Seok, K.H. Lee, H.W. Gil, J.O. Yang, E.Y. Lee and S.Y. Hong, 2009. Factors for determining survival in acute organophosphate poisoning. Korean J. Intern. Med., 24: 362-367.

Kaur, P., B. Radotra, R.W. Minz and K.D. Gill, 2007. Impaired mitochondrial energy metabolism and neuronal apoptotic cell death after chronic dichlorvos (OP) exposure in rat brain. Neurotoxicology, 28: 1208-1219.

Lifshitz, M., E. Shahak and S. Sofer, 1999. Carbamate and organophosphate poisoning in young children. Pediatr. Emerg. Care, 15: 102-103.

Moritz, F., J.M. Droy, G. Dutheil, J. Melki, G. Bonmarchand and J. Leroy, 1994. Acute pancreatitis after carbamate insecticide intoxication. Intensive Care Med., 20: 49-50.

Munidasa, U.A., I.B. Gawarammana, S.A. Kularatne, P.V. Kumarasiri and C.D. Goonasekera, 2004. Survival pattern in patients with acute organophosphate poisoning receiving intensive care. J. Toxicol. Clin. Toxicol., 42: 343-347.

Osawa, S., K. Kariyone, F. Ichihara, K. Arai, N. Takagasa and H. Ito, 2005. Development and application of serum cholinesterase activity measurement using benzoylthiocholine. Clin. Chim. Acta, 351: 65-72.

Osmundson, M., 1998. Insecticides and Pesticides. In: Emergency Toxicology, Viccellio, P. (Ed.). 2nd Edn., Lippincott-Raven Publishers, Philadelphia, PA., pp: 401-413.

Peter, J.V. and A.M. Cherian, 2000. Organic insecticides. Anaesth Intensive Care, 28: 11-21.

Pope, K. and D.K. Olson, 1990. Pesticides and their control. AAOHN J., 38: 353-359.

Saadeh, A.M., 2001. Metabolic complications of organophosphate and carbamate poisoning. Trop. Doct., 31: 149-152.

Sharma, B., 1999. Effect of carbaryl on some biochemical constituents of the blood and liver of Clarias batrachus, a fresh-water teleost. J. Toxicol. Sci., 24: 157-164.

Sungur, M. and M. Guven, 2001. Intensive care management of organophosphate insecticide poisoning. Crit. Care, 5: 211-215.

Verger, P., B. Gilles, H. Guegan and J. Agathe-Nerine, 1996. Acute pancreatitis after organophosphorus poisoning. Presse. Med., 25: 1258-1258, [Article in French].

WHO, 2001. WHO recommended classification of pesticides by Hazards and Guidelines to Classification 2000-2001. WHO/PCS/01.4, World Health Organization, Geneva.

Weizman, Z. and S. Sofer, 1992. Acute pancreatitis in children with anticholinesterase insecticide intoxication. Pediatrics, 90: 204-206.

Yamanaka, S., M. Yoshida, Y. Yamamura, M. Nishimura and Y. Takaesu, 1993. A study on acute organophosphorus poisoning-changes in the activity and isoenzyme patterns of serum cholinesterase in human poisoning. Nippon Eiseigaku Zasshi, 48: 955-965.

Yamashita, M., M. Yamashita, J. Tanaka and Y. Ando, 1997. Human mortality in organophosphate poisonings. Vet. Human Toxicol., 39: 84-85.