

Research Article



The Role of Muscle Injury in organophosphate Poisoning and Development of Intermediate Syndrome

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ABSTRACT

Organophosphate poisoning (OP) is a major global health problem with hundreds of thousands of deaths every year. (OP) poisoning leads to three main syndromes: Acute cholinergic syndrome, intermediate syndrome and delayed neuropathy. Intermediate syndrome remains a major contributor to the high morbidity and mortality in (OP) poisoning and important medical problem in the developing world. The objective of the present study is to determine whether the serum levels of the muscle enzymes can predict the subsequent development of intermediate syndrome. The development of intermediate syndrome was studied in 90 patients with acute (OP). These 90 patients were subjected to analysis of demographic data, clinical picture and laboratory analysis of the level of cholinesterase activity in serum as well as the levels of creatine kinase, creatine kinase MM, and Lactic dehydrogenase (LDH). S.G.O.T. and S.G.P.T. levels were done as well. Our results showed serum cholinesterase level ranged from 200-3500 with median 890 and a mean 1059.2 ± 645.53 . Creatine kinase ranged from 100-3088 with median 180 and a mean 315.62 ± 599.36 . Creatine kinase MM ranged from 85-3049.6 with median 160 and a mean of 295.87 ± 594.94 . Lactic dehydrogenase ranged from 198 -1290 with median 592 and a mean of 573.22 ± 258.94 . S.G.O.T. ranged from 12-45 with median 18 and a mean 22.11 ± 9.29 . S.G.P.T. ranged from 9-50 with median 20 and a mean 23.51 ± 10.15 . Our conclusion showed the serum levels of muscle enzymes can be helpful in prediction of development of intermediate syndrome.

Keywords: Cholinesterase, Intermediate syndrome, Muscle enzymes, Organophosphate poisoning.

INTRODUCTION

Organophosphate (OP) compounds are diverse group of chemicals used in both domestic and industrial settings. These (OP) pesticides are widely used in agriculture and at home.¹

They are the commonest pesticides used due to their readily available. There is widespread abuse of these compounds with suicidal intent.²

Organophosphate compounds are irreversible inhibitors of the enzyme acetyl cholinesterase (AChE), binding to the esteratic site of the enzyme. They inhibit both cholinesterase and pseudo-cholinesterase activity. This inhibition causes accumulation of acetylcholine at synapses with resultant overstimulation of neurotransmission.³ The clinical features are due to excess acetylcholine at the muscarinic and nicotinic receptors which leads to initial stimulation and eventual exhaustion of cholinergic synapses.⁴

There are three distinct phases: Acute cholinergic crisis, intermediate syndrome (IMS) and delayed polyneuropathy syndrome.⁵

The symptoms of the acute cholinergic crisis are due to stimulation of the muscarinic and nicotinic receptors. Muscarinic manifestations include excessive salivation, miosis, diarrhea, vomiting, bronchorrhoea, bronchospasm, bradycardia and urination. Nicotinic manifestations include decreased muscle power and skeletal muscle fasciculations.⁶

The cholinergic phase usually passes off within 48-72 hours but complete clinical recovery from all the effects may take up a week.

The intermediate syndrome (IMS) is a second stage of weakness occurs 1-4 days later with or without a symptom-free interval and if left unrecognized, can lead to fatal respiratory depression.⁷ It is a syndrome of muscular paralysis occurring in conscious patients and is often associated with respiratory failure.⁸

Aim of the work

The aim of this study is to investigate the pattern of development of (IMS) and to predict its occurrence by estimation of serum levels of the muscle enzymes.

SUBJECTS AND METHODS

This study was conducted to provide recent information about acute (OP) toxicity and subsequent development of (IMS) among a sector of patients attending the (NECTR), Cairo University during the period from January to December 2009. All patients were included in the current study provided a written informed consent prior to their inclusion. This study was approved by the local ethical committees and in accordance with the ethical standard laid down in the 1964 Declaration of Helisinki.

A total number of 90 patients were interviewed using a standard sheet. It included information about age, sex, residence and weight. The amount and nature of (OP) compound and the circumstances of poisoning whether accidental or intentional were studied as well. All the



patients admitted to emergency department of NECTR. Chemical analysis was performed using the manual spectro-photometer PM 750 for measurement of S.G.O.T. (AST) and S.G.P.T. (ALT) at the laboratory of Kasr El Eini ICU department of NECTR.

The level of cholinesterase enzyme and the serum levels of creatine kinase, creatine kinase MB, creatine kinase MM and lactic dehydrogenase were measured by Cobus-Integra spectrophotometer at the laboratory of Kasr El-Eini hospital Cairo University. Patients were brought to the emergency department of (NECTR) from rural areas and some urban areas after 2 hours of organophosphate ingestion. They showed frequent vomiting and diarrhea with contaminated clothes which complicate the condition by further risk of absorption through skin. Unfortunately, some cases ingest large amount of organophosphate pesticide to attempt suicide. However, children don't ingest large quantities as they took it accidentally through contaminated food. Some few cases which were referred from other hospitals had been given atropine only.

Data handling and Statistical Analysis

Data were collected, checked, revised and entered the computer. Data were analyzed by SPSS statistical package version 17. Excel computer program was used to tabulate the results, and represent it graphically.

Qualitative variables were expressed as count and percentages.

The significant difference in distribution was tested by using Chi-square-test at $p < 0.05$.

The significant difference between groups was tested by using independent t-test at $p < 0.05$.

Pearson correlation coefficient was calculated to show the power and direction of the linear relationship between the measured quantitative variables at $p < 0.05$.

Management of these patients was based on clinical examinations, laboratory findings and other investigations. Most of patients were treated by atropine, oximes, fluids after decontamination with or without activated charcoal according to the case. Severe cases with respiratory difficulty were transferred to the intensive care unit for respiratory support and they received the same previous treatment as well. Any complications observed during follow up for both groups of patients were recorded. Manifestations of (IMS) were studied carefully. Any muscle weakness especially the ocular, neck, bulbar, proximal limb and respiratory muscles were reported. Examination of the cranial nerves was done as well. The patients were under observation for 48 hours. They considered to have (IMS) if after recovery from cholinergic symptoms, new or worsening sign of proximal muscle weakness and respiratory paralysis (as evidenced by retention of carbon dioxide, failure to wean in patients on ventilator or muscle fasciculations) occurred any time after the first 12-24

hours. Characteristically, the patients cannot raise their head from pillow.

RESULTS

More than 28% of the studied group accidentally ingested organophosphate compounds whereas 72% of them took it for suicidal attempts. The poisons used varied with each individual patient but the types consumed mainly parathion and malathion. However, some patients ingest a mixture of different types of organophosphate pesticides. The amount consumed was difficult to infer as often the only clue was empty packet or bottle.

The demographic characteristics of the studied group are presented in table 1. The age was distributed as follows: 28.9% of the studied group aged from 1-10 years, whereas 31.1% aged from 10-20 years and 22.2% aged from 20-30 years. However, 17.8% of them were older than 30 years. Males represented 33.3% of patients whereas 66.7% were females. Cairo provided 42.2% of the studied group and Giza provided 48.9% of them. However, rural areas provided 8.9% of cases. Clinically 60% of patients admitted with vomiting, diarrhea, abdominal cramps, salivation, lacrimation, bradycardia, hypotension, miosis, bronchospasm and bronchorhea. 40% of cases admitted with severe manifestations and underwent mechanical ventilation for acute respiratory failure as evidenced by arterial blood gases (A.B.G.) findings.

Patients were considered to have (IMS) when they showed overt signs of it after recovery from cholinergic crisis. They are 18 patients (20%) out the total number 90 and they are distributed as follows: 8 patients were incubated in the ICU after a varying period of 4-24 hours and 10 of the 18 patients self-extubated on day 3 of ventilation and needed to be re-incubated and then necessitating full ventilator support.

Table 2: Showed descriptive statistics of cholinesterase enzyme level where it was severely inhibited in the first measure and ranged from 200-3500 with median 890 and a mean 1059.20.

2nd measure of cholinesterase enzyme showed improvement with a range between 1356-6270 and median 1880 and a mean 1982.82. 3rd measure showed complete recovery, however, some patients had a level of 1030 (normal range up to 4300 U/L) in spite of clinical improvement.

The same table showed liver enzymes where S.G.O.T. level ranged from 12-45 with median 18 and a mean 22.11 and S.G.P.T. level ranged from 9-50 with median 20 and a mean 23.51.

Muscle enzymes level reported in the same table where creatine kinase ranged from 100-3088 with median 180 and a mean 315.62 and creatine kinase MM ranged from 85-3049.6 with median 160 and a mean 295.87 while lactic dehydrogenase ranged from 198-1290 with median 592 and a mean 573.22.



Table 3: Showed that acetyl cholinesterase was inhibited > 75% during admission (1st measure). Also, there are the percentages of increase of muscle enzymes e.g: creatinine kinase and lactic dehydrogenase.

Table 4 and figure 1, 2: Showed a comparison between the two groups of patients as regards the level of cholinesterase, liver and muscle enzymes. It showed a statistically significant difference between the severely poisoned patient with delayed onset of medical interference and mild cases as regards all parameters measures.

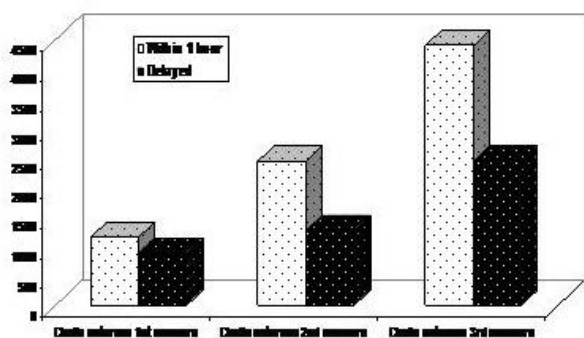


Figure 1: Effect of delayed onset of medical interference on Cholinesterase

Table 1: Distribution of Age, sex and residence of the studied group

Category		Count	%
Age	1 y - <10 y	26	28.9
	10 - < 20 y	28	31.1
	20 - <30	20	22.2
	30 and more	16	17.8
Sex	Male	30	33.3
	Female	60	66.7
Residence	Cairo	38	42.2
	Giza	44	48.9
	Fauom	6	6.7
	Assiut	2	2.2
Total		90	100.0

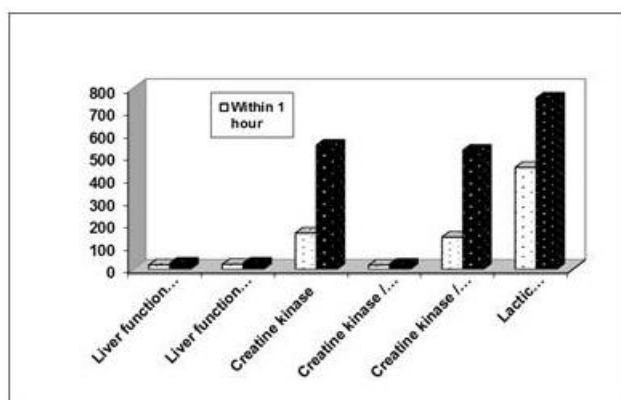


Figure 2: Effect of delayed onset of medical interference on liver function tests and muscle enzymes

DISCUSSION

Organophosphate (OP) compounds are chemical agents in widespread use through the world.

There are no rules and regulations governing the purchase of these products and they are therefore readily available "over the counter", although there are a major cause of morbidity and mortality. Exposure to (OP) in an attempt to commit suicide is a key problem, particularly in the developing countries. It is a more common cause of poisoning than the chronic exposure experienced by farmers or sprayers in contact with pesticides.⁹

Acute (OP) insecticide poisoning can manifest 3 different phases of toxic effects namely, acute cholinergic crisis, (IMS), and delayed polyneuropathy.¹⁰ The proposed mechanisms of (IMS) include different susceptibility of various cholinergic receptors, muscle necrosis, prolonged acetyl cholinesterase inhibition, inadequate oxime therapy, down regulation or desensitization of post synaptic acetylcholine receptors, failure of postsynaptic acetylcholine release and oxidative stress-related myopathy.¹¹

Long term exposure to (OP) was associated with subclinical possible neuropathies.¹²

The clinical manifestations of (IMS) typically occur within 24 to 96 hours, affecting conscious patients without cholinergic signs and involve the muscles of respiration, proximal limb muscles, neck flexors and muscles innervated by motor cranial nerves. Patients with atypical manifestations of (IMS), especially a relapse or continuum of acute cholinergic crisis were frequently reported in clinical studies of (IMS).¹³

These data in accordance of our findings in which all cases admitted with cholinergic crisis. 18 patients (20%) of cases developed (IMS) with the course of cholinergic manifestations and 10 out of 18 were re-incubated and put on a ventilator due to respiratory muscle weakness and seem to be a continuum of acute cholinergic crisis (Atypical IMS).

Clinical features of IMS first termed by Wadia et al as type II paralysis, and characterized by muscle paralysis following the acute cholinergic phase and this is typically occurred (in 8 out of 18) patients developed IMS, after recovery from cholinergic phase.¹⁴

IMS which is considered a second stage of weakness occurs 1-4 days after acute cholinergic phase with or without symptom free interval can lead to fatal respiratory depression. In this condition, mechanical ventilation is mandatory.¹⁵ All these data emphasize our results and findings.

Senel et al reported that IMS occurs in patients with severe organophosphate poisoning.¹⁶

The conclusions derived by Sedwick and Senanayake and many experimental and clinical studies reported that, IMS relates to the severity of poisoning not the specific (OP)

and to prolonged inhibition of acetyl cholinesterase activity of the erythrocytes, brain and muscle end plate with pre and post synaptic impairment of neuromuscular

transmission. Some authors propose that poor regulation of acetylcholine receptors could explain that syndrome and neurophysiological findings.¹⁷⁻¹⁸

Table 2: Descriptive statistics of cholinesterase, liver function tests and muscle enzymes of the studied group

Measure	N	Minimum	Maximum	Median	Mean	S.D
Cholinesterase 1	90	200	3500	890	1059.20	645.53
Cholinesterase 2	90	356	6270	1880	1982.82	1138.62
Cholinesterase 3	90	1030	7600	3300	3628.24	1730.83
Liver function (SGOT)	90	12	45	18	22.11	9.29
Liver function (SGPT)	90	9	50	20	23.51	10.15
Creatine kinase	90	100	3088	180	315.62	599.36
Creatine kinase / MB	90	10	40	20	19.76	5.74
Creatine kinase / MM	90	85	3049.6	160	295.87	594.94
Lactic dehydrogenase	90	198	1290	592	573.22	258.94

Table 3: Percentages of abnormalities of cholinesterase and muscle enzymes of the studied group

Measure	Normal	Patient Mean± S.D	% of Abnormality
Cholinesterase (% of inhibition from)	up to 4300	1059.20±645.53	-75.37
Creatine kinase (% of increase)	25 - 135 U/L	390.00±725.64	188.89
Creatine kinase / MB (% of increase)	Up to 16 U/L	19.76±5.74	23.47
Creatine kinase / MM	Up to 8 U/L	295.87±594.94	3598.36
Lactic dehydrogenase (% of increase)	Up to 480 U/L	573.22±258.94	19.42

Table 4: Effect of delayed onset of medical interference and severity of manifestations on Cholinesterase enzymes, liver function tests and muscle enzymes

Measure	Within 1 hour (n=36) Mild	Delayed (n=54) Severe	t-value	P-value
	Mean ± S.D	Mean ± S.D		
Cholinesterase 1 st measure	1169.74±726.69	893.39±471.53	1.423	0.162
Cholinesterase 2 nd measure	2459.07±1150.39	1268.44±656.45	3.972	0.000*
Cholinesterase 3 rd measure	4411.26±1670.47	2453.72±1025.44	4.436	0.000*
Liver function (SGOT)	19.00±8.21	26.78±9.03	2.991	0.005*
Liver function (SGPT)	21.44±8.21	26.61±12.12	1.708	0.095
Creatine kinase	160.00±37.37	549.06±911.86	2.227	0.031*
Creatine kinase / MB	17.95±4.57	22.47±6.36	2.776	0.008*
Creatine kinase / MM	142.05±33.95	526.59±905.76	2.217	0.032*
Lactic dehydrogenase	450.48±178.13	757.33±255.34	4.756	0.000*

S.D. = Standard Deviation, * = There is a significant difference between groups by using independent t-test at p<0.05

It is possible that several other factors such as age of patients, duration of initial intoxication could contribute to the differences in the results of neurophysiological monitoring.¹⁹ They conclude that IMS is a spectrum disorder progresses over time through a series of changes which result in respiratory failure among the most severe cases.

All these data and findings support our results in which there were 40 %of cases admitted with severe poisoning and needed assisted ventilation, and 20 %developed IMS.

Acetyl cholinesterase is present in 2 forms: True acetylcholinestase which is found primarily in the tissues

and erythrocytes, and pseudo cholinesterase which is found in the serum and liver.

(OP) compounds are acid-transferring inhibitors of cholinesterase. They cause cholinesterase to become firmly (and sometimes irreversibly) phosphorylated. This means that the action of cholinesterase will be inhibited. Cleavage of the carbon-enzyme bond from acetylcholine is complete in a few microseconds.

However, the breaking of the phosphorus-enzyme bond requires a period varying from 60 minutes to several weeks, depending on the (OP) compound involved. Reactivation of the inhibited enzyme may occur



spontaneously.²⁰ The rate of reactivation will depend on species, the tissue, and chemical group attached to the enzyme.

Acetyl cholinesterase was inhibited > 90 %through the course of poisoning.²¹ The same results obtained in the present study as cholinesterase is inhibited > 75% (table 3).

Reactivation may be enhanced by hydrolysis of the acid-radical-enzyme through the use of oximes i.e .reactivating agents.²² All these findings emphasize our results in which cholinesterase enzyme was inhibited especially on admission (the first measure) with improvement in the 2nd and 3rd measures (table 2). Response to reactivating agents decline with time, this process being caused by "ageing" of the inhibited enzyme.²³ These data are in accordance of our findings of the present study in which 40 %of cases admitted with severe poisoning and late onset of medical interference.

Muscle injury was seen in all patients at the beginning of admission in a study done by John et al.²⁴ They documented that profiles of blood muscle isoenzymes showed significantly greater muscle injury in those patients with greater severity of poisoning at admission and those who developed IMS. These data typically emphasize our results in which creatine kinase and creatine kinase MM and lactic dehydrogenase are higher than normal (table 2) and the percentage of their increase indicative of muscle injury (table 3).

In contrast Aygun et al. found that there was no significant difference in initial serum levels of creatine kinase and (S.G.O.T) between the patients with severe poisoning and mild poisoning.²⁵ Also they found that there was no difference in initial serum levels of both enzymes between the patients with and without IMS . However, in our study we found that there is a statistically significant difference between the patients with severe poisoning and mild poisoning as regards cholinesterase level, S.G.O.T, creatine kinase MM and lactic dehydrogenase (table 4).

Yang and Deng had documented a case with inhibited cholinesterase enzyme and a high level of creatine kinase which support our results.²⁶

Several researchers stated that oxidative cellular damage to muscle membranes could be another possible mechanism of muscle necrosis.^{11,27}

Our protocol of treatment in the (NECTR) coincides with that of Chen in which he documented that therapy with obidoxime, a more potent oxime, significantly decreased the incidence of respiratory failure and the length of hospitalization (table 6).²⁸

Repeated doses of fresh frozen plasma therapy has been proposed in the treatment of IMS. Guven et al found that frozen plasma therapy could prevent the development of IMS and related mortality through the restoration of plasma acetyl cholinesterase.²⁹

However, further studies on a large scale population are suggested and repetitive nerve stimulation (RNS) and Electromyography (EMG) may be helpful in further studies.

CONCLUSION

Although IMS generally occurred among patients with prolonged and severe inhibition of acetyl cholinesterase, not every patient with severe inhibition of the enzyme developed IMS .There were other risk factors of IMS as well, including delayed metabolism of (OP) or impaired organ function, severity of poisoning, elevated muscle enzymes and inadequate or late oxime therapy.

The serum levels of muscle enzymes can be helpful in prediction of development of IMS.

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