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Serum Enzymes in Organophosphorous Poisoning

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Abstract
Organophosphorus compounds have been widely used for a few decades in agriculture for crop protection and pest control. In India Organophosphorus poisoning is the most common. Acetylcholinesterase is an enzyme that breaks down the neurotransmitter acetylcholine results in a lowered level of acetylcholine, and ultimately the termination of nerve impulses. Organophosphorous compounds covalently block the active site of serine residue of acetylcholinesterase this irreversible inactivation leads to an excess accumulation of acetylcholines in the peripheral and central nervous system causing cholinergic manifestations.
We have estimated the serum enzymes such as acetylcholinesterase, lactate dehydrogenase, creatine kinase, amylase and lipase in organophosphorus poisoning patients and healthy controls. We found significantly decreased acetylcholinesterase ($p<0.001$) and significantly increased lactate dehydrogenase, creatine kinase, amylase and lipase in patients with organophosphorus poisoning as compared to controls ($p<0.001$). Serum acetylcholinesterase level was estimated only at the time of admission. Daily estimation of acetylcholinesterase and amylase levels may provide more informative in prediction the prognosis or severity of poisoning and to monitor the effectiveness of therapy.
Key words: *Acetylcholinesterase, Lactate dehydrogenase, Creatine kinase Amylase*

Introduction

Organophosphorus [OP] compounds have been widely used for a few decades in agriculture for crop protection and pest control. Some have also been used in the medical treatment of myasthenia gravis, e.g. diisopropyl phosphorofluoridate [DFP], tetraethyl pyrophosphate [TEPP], and octomethyl pyrophosphotetramide [OMPA]. Some OP esters are still used to treat glaucoma [Ecothiopate]. In addition to these beneficial agricultural, veterinary, and medical uses, some highly potent OP anticholinesterase compounds, including tabun, sarin and soman, have been used as "nerve gases" in chemical warfare. They are also been used as plasticizers, stabilizers in lubricating and hydraulic oils, flame retardants, and gasoline additives [(1, 2)].

In India Organophosphorus poisoning is the most common [(3)]. Organophosphorus poisoning compounds inhibit acetylcholinesterase at

neuromuscular junction, in autonomic and central nervous system resulting in accumulation of acetylcholine [ACh] and over stimulation of ACh receptors resulting in acute cholinergic crisis which is characterized by bradycardia, increased gastrointestinal motility, emesis, sweating, tachypnoea, salivation, lacrimation, altered sensorium, fasciculation, bronchospasm, blurred vision and urination. The complications include acidosis, respiratory paralysis, acute renal failure, seizures, arrhythmia, aspiration etc and death may be due to combination of one or above complications [(4)].

Acetylcholinesterase (AChE) is an enzyme that breaks down the neurotransmitter acetylcholine. 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 [(5)].

Elevations are seen in Serum lactate dehydrogenase (LDH) and creatine kinase (CK) activities following acute OP poisoning as a result of muscle injury [(6)]. LDH is an enzyme that catalyzes the interconversion of lactic acid and pyruvic acid. It is a hydrogen transfer enzyme that uses the coenzyme NAD^+. LDH is widely distributed in the body [(7)].

Creatine kinase (CK) also known as creatine phosphokinase (CPK) or phosphocreatine kinase, is an enzyme expressed by various tissues and cell types. CK catalyses the interconversion of phosphocreatine (or creatine phosphate) to creatine [(8)].

Amylase is a digestive enzyme that cleaves starch into smaller carbohydrate groups and finally into monosaccharides, by hydrolysis of internal alpha-1, 4-glycoside bonds, which results in the production of maltose and oligosaccharides. Lipase is an enzyme that catalyzes the hydrolysis of fats [(9)].

The present study was planed to examine alterations in enzymes such as acetylcholine-esterase (AChE), lactate dehydrogenase (LDH), creatine kinase (CK), amylase and lipase in organophosphorus poisoning patients.

Materials and Methods

The present study was carried out in the Department of Biochemistry, Government Medical College and Hospital, Miraj (Maharashtra, India). Study protocol was approved by ethical committee, Government Medical College. Miraj.

Sample size

The study group includes total 80 subjects. This includes patients as well as control.

Patients: Total 40 patients with organophosphorus poisoning hospitalized at Government Medical College and Hospital. The diagnosis of the patient was done by the clinicians on the basis of the patient's condition, smell of the Organophosphorus poisoning compound, clinical history, personal history, physical examination.

Control: The 40 healthy controls were taken in all age group with both genders attending the OPD of Government Medical College and Hospital, Miraj during the same period.

Previous history of accidental or suicidal poisoning and no any abnormal clinical findings, particularly in the context of metabolic and nutritional disorders were excluded from the study.

Blood Collection:

Informed consent was obtained from the participants. Blood samples were collected from 40 patients immediately on admission in plain bulb.

In addition blood samples were also obtained from 40 healthy age sex matched individuals to serve as controls.

Blood samples from plain bulb were centrifuged and clear serum were separated and used for estimation of Cholinesterase, Lactate Dehydrogenase (LDH) and Creatine Kinase (CK).

Serum cholinesterase was estimated by kinetic butyrylthiocholine kit [(10)], lactate dehydrogenase

by SCE recommended [11,12], Creatine Kinase by liquid stable optimized UV method [13], amylase by direct substrate method [14] and lipase by Turbidimetric U. V. Method [15] and levels were expressed as U/L. The data were evaluated statistically by using student 't' and 'F' test, 'F' value was calculated by Minitab and SPSS software.

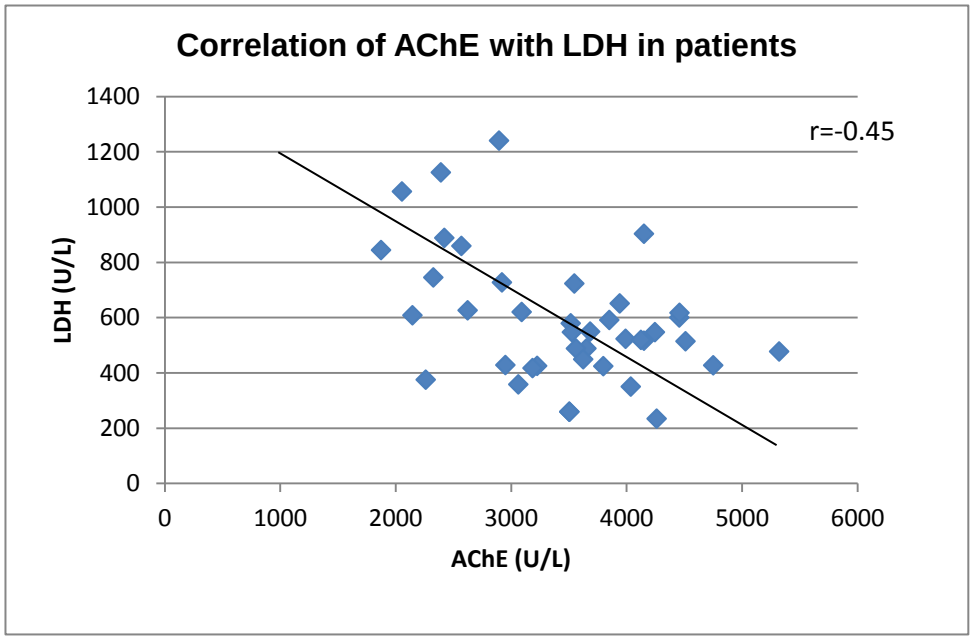
Results

We found significant decrease in AChE and significant increase in LDH, CK, amylase and lipase in patients as compared to control ($p<0.001$) (Table No.1).

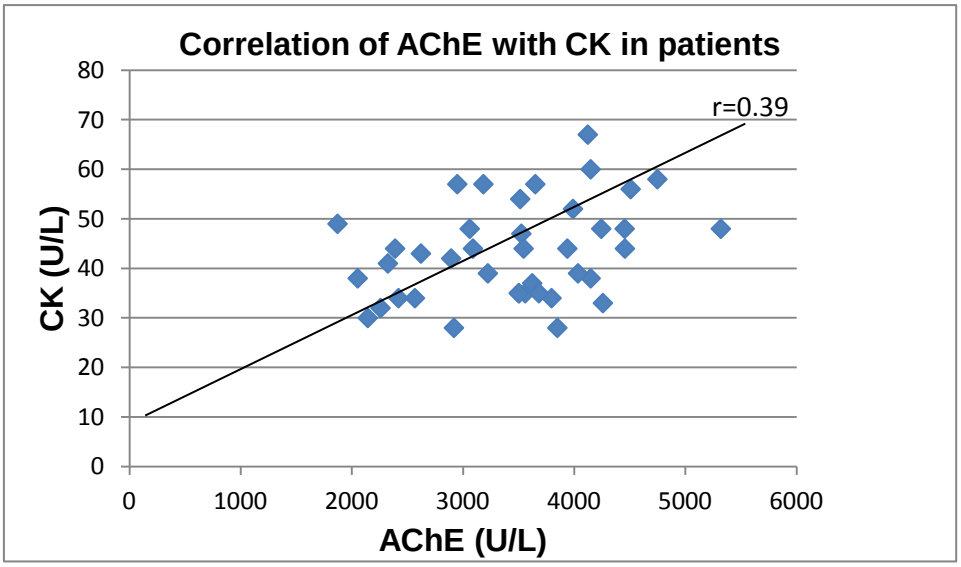
The mean values of Serum Cholinesterase (AChE) and CK in different age group are given in table no. 2. We found significant trend AChE and CK in all age groups except age above 60 years. However we found significant trend in LDH activity only in age group 41- 60 years.

We found significant trend in amylase activity only in age group 21- 40 and 41- 60 years, whereas non significant difference in lipase activity with respect to age group (Table no. 3).

Graph No. 1: Correlation of AChE with LDH in patients



Graph No. 2: Correlation of AChE with CK in patients



Graph No. 1: Correlation of Lipase with Amylase in patients

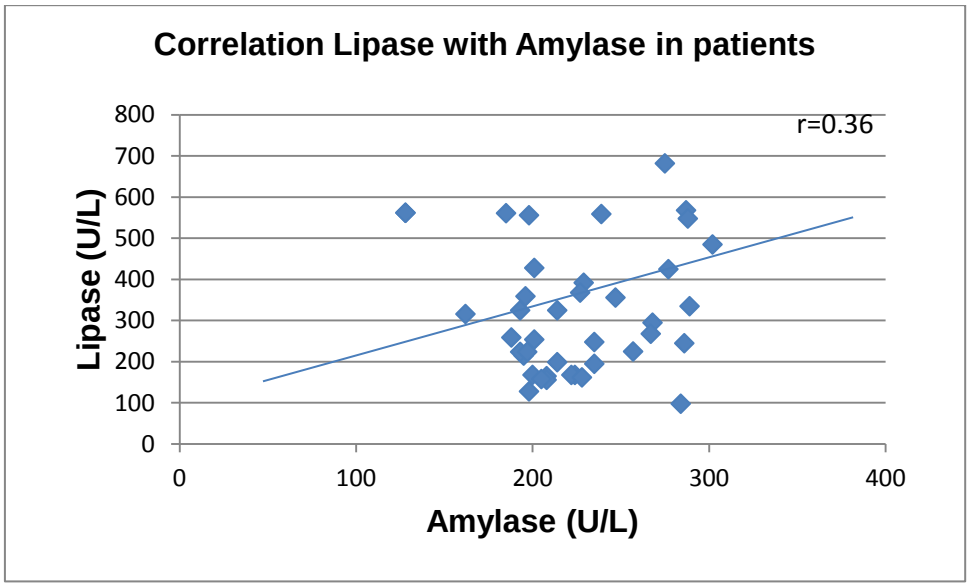


Table No. 1: Serum enzymes in OP poisoning patients and controls

Parameters	**Patients(n=40) (Mean ± SD)**	**Control (n=40) (Mean ± SD)**
Serum Cholinesterase(U/L)	3453.67* ± 817.79	8722.22 ± 3018.59
Serum Lactate dehydrogenase (U/L)	590.38* ± 228.58	373.92 ± 107.68
Serum Creatine Phosphokinase(U/L)	43.40* ± 9.69	20.80 ± 5.00
Serum Amylase(U/L)	224.45 ± 42.54	122.88 ± 39.41
Serum Lipase(U/L)	323.35* ± 155.34	162.45 ± 37.38

*p<0.001, Highly Significant

Table No.2: Serum enzymes in OPP patients

Age group (In years)	**Patients**				**Control**			
	N	**AChE(U/L)**	**LDH(U/L)**	**CK(U/L)**	**N**	**AChE(U/L)**	**LDH(U/L)**	**CK(U/L)**
Up to 20	04	3288.25* ± 278.51	418.50^{NS}± 220.67	34.25* ±4.573	02	9835.5 ± 4621.5	275.00 ±50.00	24.00 ±05.00
21 to 40	23	3514.14* ± 818.22	597.23^{NS} ±245.53	46.23* ±9.284	17	8991.78 ±3512.21	394.00 ±104.26	21.00 ±4.947
41 to 60	09	3015.60* ± 723.26	673.60* ±210.61	38.60* ±6.899	16	8070.94 ±2444.62	375.75 ±96.605	21.81 ±5.282
Above 60	04	4381.75^{NS} ± 713.8	516.50^{NS} ±73.528	49.00^{NS} ±12.832	05	8689.00 ±1938.18	325.60 ±146.83	17.20 ±3.768

* p<0.001, Highly Significant, NS= Non significant

Table No.3: Serum enzymes in OPP patients

Age group (In years)	Patients			Control		
	n	Amylase (Mean ± SD) (U/L)	Lipase (Mean ± SD) (U/L)	n	Amylase (Mean ± SD) (U/L)	Lipase (Mean ± SD) (U/L)
Up to 20	04	203.5^{NS} ± 10.5	443.50^{NS} ± 136.83	02	156.25 ± 11.42	121.0 ± 21.0
21 to 40	23	226.56* ± 34.097	309.41^{NS} ± 164.81	17	125.86 ± 45.174	159.83 ± 35.69
41 to 60	09	213.94* ± 50.046	301.90^{NS} ± 131.08	16	105.30 ± 25.487	163.62 ± 38.54
Above 60	04	256.8^{NS} ± 35.94	333.50^{NS} ± 175.95	05	116.5 ± 33.92	180.60 ± 35.004

* p<0.001, Highly Significant, NS= Non significant

Discussion

We investigated the levels of AChE in OPP patients and healthy controls and are given in table no. 1. The mean value of serum AChE was decreased significantly in patients as compared to control (p<0.001). This may be due the inhibition of AChE by organophosphate compounds.

OP compounds cause irreversible inhibition of acetylcholine esterase and create symptoms collectively referred to as cholinergic crisis. This is due to the accumulation of acetylcholine at the synapse which over stimulates the central and the peripheral nervous system. The resulting muscarinic and nicotinic symptoms usually continue for days and months until the acetylcholine esterase enzyme forms again [(4)].

Organophosphates bind and inhibit cholinesterase (ChE) and their acute toxicity manifests as a cholinergic crisis with excessive glandular secretions, altered mental status, and weakness. Cholinesterase activity correlates well with the amount of pesticides absorbed in the organism and its inhibition level is also related with toxic manifestations in the body. The mechanism by which OP compounds induce cardio-toxicity is still uncertain. The cardiac toxicity associated with OP poisoning is caused by more than one mechanism. Possible mechanisms include sympathetic and parasympathetic over-activity, hypoxemia, acidosis, electrolyte derangements and a direct toxic effect of the compounds on the myocardium [(16)].

The normal function of AChE is to terminate neurotransmission due to acetylcholine, liberated at cholinergic nerve ending in response to nervous stimuli. Loss of AchE activity may lead to a range of effects resulting from excessive nervous stimulation and culminating in respiratory failure and death [(17)].

OP compounds are acid-transferring inhibitors of cholinesterase. They cause cholinesterase to become phosphorylated leads to inhibition of action of cholinesterase [(18)].

Similar findings of depletion in AChE level has been also reported in earlier studies in organophosphorus poisoning patients. This may be useful serum marker for monitoring such patients [(17, 19)].

The mean values of AChE activity with respect to age groups were given in table no. 2. We found significant (p<0.001) trend in AChE activity in all age groups except above 60 years in patients. In age group 41-60 years AChE activity was low as compared to other age groups, as the age increases

AChE activity goes on increasing. We observed increase in results with increase in age exception 41-60 years. We found highest value in age group above 60 years.

Table no. 1 shows serum LDH activity in OPP patients and control. We found significantly increased ($p<0.001$) LDH this may be due increased anaerobic glycolysis. LDH enzyme system plays principal role in the glycolytic cycle in the cell for conservation of stored energy (pyruvate or lactate), this enzyme released by injury to different tissues [17].

Increased serum LDH activity in poisoning cases indicating muscular functional impairment due to OP toxicity. LDH activity is directly linked with the glucose metabolism. It is widely distributed enzyme found in all organs of our body [16].

Muscle injury was seen in patients with organiphosphorous poisoning at the beginning of admission. Profiles of blood muscle isoenzymes showed significantly greater muscle injury in those patients with greater severity of poisoning at admission [18].

Our result is similar to those obtained by Agarwal et al [16], S. Hariprasad et al [17] and W Nashwa et al [18] who have found increased LDH activity in serum of patients with organophosphorus poisoning.

Table No. 2 shows the serum LDH activities in different age groups. We found highest LDH value in age group 41-60 years and lowest in age group less than 20 years in patients. We found significant negative correlation ($r= -0.45$) in between AChE and LDH (Graph no. 1).

The mean value of serum CK was increase significantly in patients as compared to control ($p<0.001$) (table no. 1) indicating muscular functional impairment due to OP toxicity [16].

The intake of insecticide followed by vomiting causes hypertension and puts excessive stress on heart. As a result the heart beat increases to a large extent. This may result in the elevation of CK and CKMB [16].

Sen R etal (6) showed serum Cholinesterase serves as a diagnostic parameter for organophosphorus poisoning and correlates with the severity but it cannot be used as a prognostic biomarker. They showed strong degree of positive correlation of CK with the severity of poisoning and can be used as a predictor of outcome in organophosphorus poisoning.

Table No. 2 shows that the activities of CPK different age groups. We found significant trend in all age group except above 60 years in patients. We found highest CK level in age group above 60 years and lowest in age group less than 20 years. We found significant positive correlation ($r= 0.39$) in between AChE and CK (Graph No. 2).

We found significantly ($p<0.001$) increased levels of amylase and lipase in OPP patients as compared to control (table no. 1). Acute pancreatitis as a complication of OP insecticide poisoning is a result of acetylcholine release from pancreatic nerves and prolonged hyperstimulation of pancreatic acinar cells. This hyperstimulation is thought to be the mechanism of acute pancreatitis after poisoning by OP insecticide [20]

S. Panda et al [4] showed significant risein amylase with severity of the degree of intoxication. The findings of their study showed usefulness of biochemical and clinical indices in the management of organophosphorous poisoning thereby recognizing the complications early and facilitating early management.

Table No.3 shows that the activities of serum amylase and serum lipase in different age groups. We found highest amylase and lipase in age group above 60 years whereas lowest amylase in age group less than 20 years and lipase in age group 41-60 years in patients.

We observed significant trend in serum amylase activity in age group 21-40 and 41-60 years, however the non significant trend in serum lipase activity in OPP patients.

We found positive significant correlation ($r= 0.36$) in between serum lipase and amylase in patients with OP poisoning (Graph No.3). These two enzymes synthesized and secreted pancreas.

Conclusions

This study is limited by the facts that sample size was small. We estimated all parameters only at the

day of admission. Daily estimation of AChE CK, LDH and amylase levels may provide more informative in prediction the prognosis or severity of poisoning and to monitor the effectiveness of therapy.

We conclude that the serum AChE is reduced in OP poisoning and serum AChE level should be estimated routinely in all cases of OP poisoning because it has prognostic significance. Estimation of serum amylase may be useful for the early detection of complication such as pancreatitis after OP poisoning.

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