ORIGINAL ARTICLE

Study of Serum Amylase and Serum Cholinesterase in Organophosphorus Poisoning

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Abstract:

Background: Poisoning due to organophosphorus compounds is most commonly seen. Earlier plasma cholinesterase level was used to assess the severity of poisoning. Presently serum amylase is being recommended as a better indicator of severity. *Aims and Objectives:* To study plasma cholinesterase and serum amylase levels in acute organophosphorus and to correlate serum amylase levels with clinical severity and outcome. *Material and Methods*: A total of 80 patients in the study admitted to a tertiary care centre within 24 hours with a history of organophosphorus poisoning were included in study. Estimation of plasma cholinesterase and serum amylase was done at the time of admission, and on 3rd day and on 5th day. *Results:* Occurrence of organophosphorus poisoning was more common among age group 21-30 years and among males (57.5%). They were 25 (31.2%) farmers, 23 (28.8%) students, and 22 (27.5%) housewives. Monocrotophos (45.0%) was commonly used compound. Mean value of plasma cholinesterase and serum amylase at admission are 3693 U/L, and 185.4 U/L. There was significant inhibition of plasma cholinesterase and elevation of serum amylase at admission with return to normal values on 5th day. *Conclusion:* Plasma cholinesterase inhibition <10% is associated with high degree of mortality. Hyperamylasemia >200 U/L has been associated with poor prognosis and proneness to respiratory failure.

Keywords: Amylase, Organophosphorus, Cholinesterase,

Introduction:

During past four decades more than 50,000 organophosphorous compounds have been synthesized and tested for insecticidal activity, but the number actually used for this purpose today probably does not exceed three dozen [1]. Organophosphorus (OP) compounds are commonly used as pesticides. Pesticides are designed to kill various pests. They are used in most countries around the world to protect agricultural and horticultural crops against damage. They are used at home and at work to assure a pestfree environment. Insecticides are the most commonly encountered pesticides in the developing countries; herbicides are more commonly encountered in developed countries. The importance of pesticides in India can be understood from fact that agriculture is major component of Indian economy. Incidence of poisoning by pesticides and consequent admission to the hospital has been increasing in recent decades [2]. OP ranks the foremost in the list of agents which cause acute pesticide poisoning. Causes of poisoning are suicidal, accidental and homicidal [2]. Suicidal poisoning is the most common cause in developing countries because of its cheapness and easy availability in the market. The ease of access and socio cultural factors plays important roles in the choice of organophosphorus compound as self poison [3]. OP compounds inhibit the enzyme acetyl

cholinesterase leading to accumulation of acetyl choline, which binds to muscarinic and nicotinic receptors throughout nervous system. Signs and symptoms of OP poisoning are due to persistent acetylcholine hyperstimulation at muscarinic and nicotinic receptor sites [4]. There are two forms of cholinesterases, one is true cholinesterase or acetyl cholinesterase which it is located in the erythrocytes, neuromuscular junctions and grey matter of brain, while another one is pseudocholinesterase or plasma cholinesterase which is synthesized by the liver and found in plasma, pancreas, heart and brain. Both these types of enzymes are inhibited by insecticide poisoning [5]. Numbers of studies have been done correlating serum amylase and organophosphorus poisoning. No conclusion has been derived as to whether serum amylase levels could be used along with cholinesterase levels for monitoring OP poisoning.

Material and Methods:

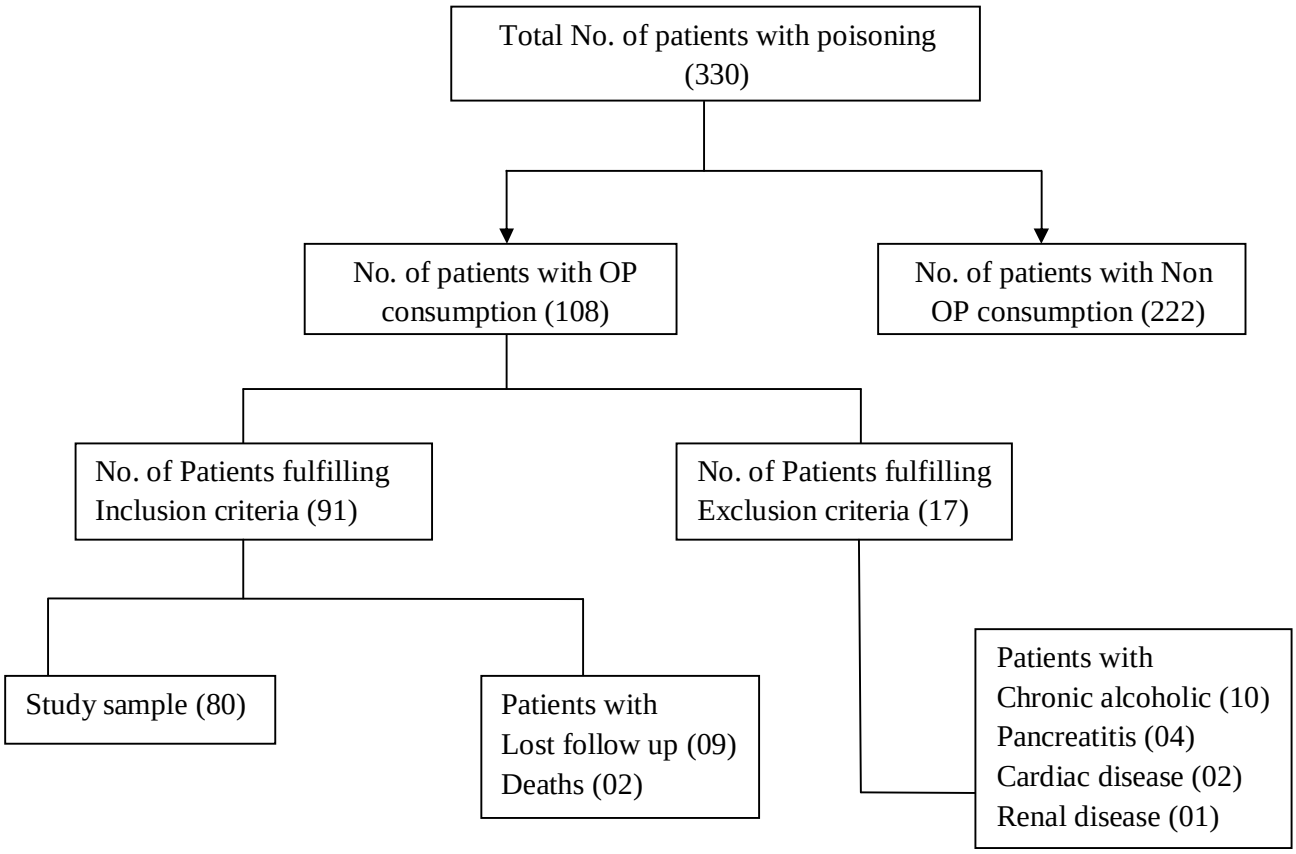
The present study was carried out in Department of Medicine, Sri B. M. Patil Medical College, Vijayapur, Karnataka. This study was done from December 2013 to June 2015. All eligible patients admitted to the hospital within 24 hours of history of OP poisoning were included in the study. Patients with chronic alcoholism, history of pancreatitis, disease of salivary glands, associated cardiac, renal, hepatic or metabolic diseases; neuromuscular diseases, women on oral contraceptives and pregnant women were excluded from the study. Presumptive diagnosis of organophosphorus poisoning was based on history, circumstantial evidence and characteristic clinical features. Gastric lavage contents were sent for analysis to poison detection centre.

Blood samples for serum amylase and plasma cholinesterase were collected at time of admission, and on 3rd day and 5th day and processed in fully automated auto analyzer. Serum cholinesterase was estimated by using new Deutsche Gesellschaft fur Klinische Chemie (DGKC) method which it has high linearity and high sensitivity. Cholinesterase catalyzes the hydrolysis of butyrlthiocholine substrate forming butyrate and thiocholine. Thiocholine reduces hexacyanoferrate (3) to hexacyanoferrate (2). The decrease of absorbance is proportional to the activity of cholinesterase in the sample. The serum was mixed with the reagent and incubated at 37C; change in the absorbance per 30 seconds during 90 seconds was measured [6-8]. Serum amylase was estimated by using 2-Chloro-4-Nitrophenyl-α- Maltotrioside (CNPG3) method in which amylase is reduced 5CNPG3 to 2-Chloro-4-Nitrophenyl-α-Maltoside. The serum was mixed with the reagent and incubated for 1 min at 37^{0}C. Change in the absorbance per minute during 3 minutes was measured [9, 10]. Other investigations done were random blood glucose estimation, serum electrolytes (sodium and potassium), blood urea, serum creatinine, complete blood picture, chest radiograph, electrocardiogram, HIV, HBs Ag, liver function tests and ABG (if necessary). Statistical data was analyzed by using Mean ± SD, correlation coefficient and chi- square test. This study was approved by the Institutional Ethics Committee. Written consent from patients or next of kin was taken.

Results:

A total of 330 patients were recruited in the study, final study sample was 80 patients after applying inclusion and exclusion criteria.

Flow Chart showing Distribution of OP Poisoning



In the study out of 80 patients, 46(57.5%) were males and 34(42.5%) were females. Male: female ratio was 1.3: 1 (Table 1). In our study, the incidence of OP was more among the age group 21-30 years; 30 (37.5%), (Table 1). There were 25 (31.2%) farmers, 23 (28.8%) students and 22 (27.5%) housewives (Fig.1). Among the symptoms, vomiting was the most common symptom i.e. 60(75.0%) out of 80.

The maximum number of cases were due to suicidal poisoning i.e. 75(93.4%), (Table 2) and the most commonly used OP compounds were monocrotophos 36 (45.0%), chlorpyrifos 12 (15.0%), (Fig. 2). A total of 24 patients required ventilator support, out of them 15 patients had Monocrotophos poisoning, five patients had chlorpyrifos poisoning and one patient each from other group of poisons.

The maximum plasma cholinesterase level at admission was 9800 U/L. while minimum level was 80 units. The overall mean plasma cholinesterase level at admission was 3369 U/L. Mean plasma cholinesterase level on 3rd day was 3781.3 U/L and on 5th day was 5271.6 U/L. This increasing trend in the cholinesterase was statistically significant ($p<0.001$) (Table 3).

The minimum serum amylase level at admission was 27 U/L while maximum level was 714 U/L. The overall mean serum amylase level at admission was 196.6 U/L which decreased to 159.3 U/L on 3rd day and to 100.6 U/L was 5th day. This decreasing trend over period of time was also statistically significant ($p<0.001$) (Table 3).

Table 1: Age and Sex Distribution

Age (Years)	Total		Male		Female	
	N	Percent	N	Percent	N	Percent
15-20	22	27.5	10	12.5	12	15.0
21-30	30	37.5	18	22.5	12	15.0
31-45	19	23.8	12	15.0	07	8.75
46-60	04	05.0	03	3.75	01	1.25
>60	05	06.2	03	3.75	02	2.50
Total	**80**	**100**	**46**	**57.5**	**34**	**42.5**

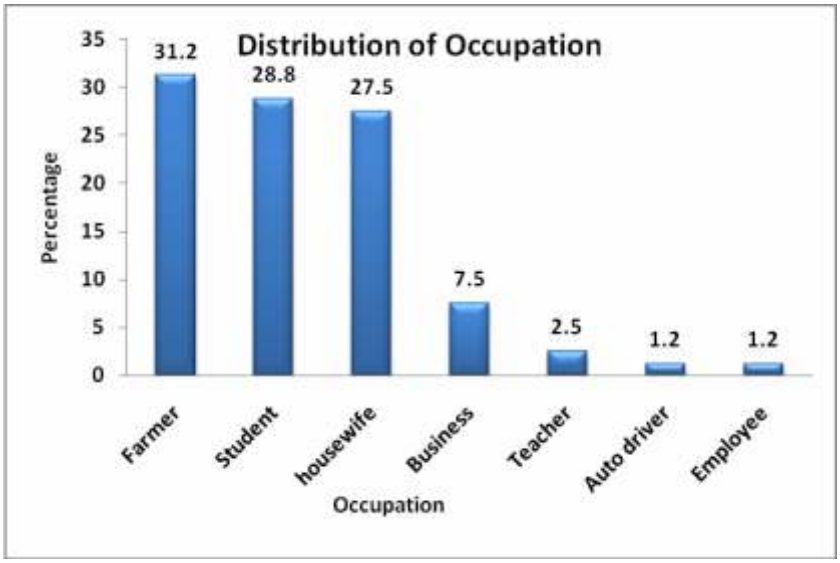


Fig. 1: Distribution of Occupation

Table 2: Nature of Poisoning

Nature of Poisoning	N	Percentage
Suicidal	75	93.4
Accidental	05	06.6
Total	**80**	**100**

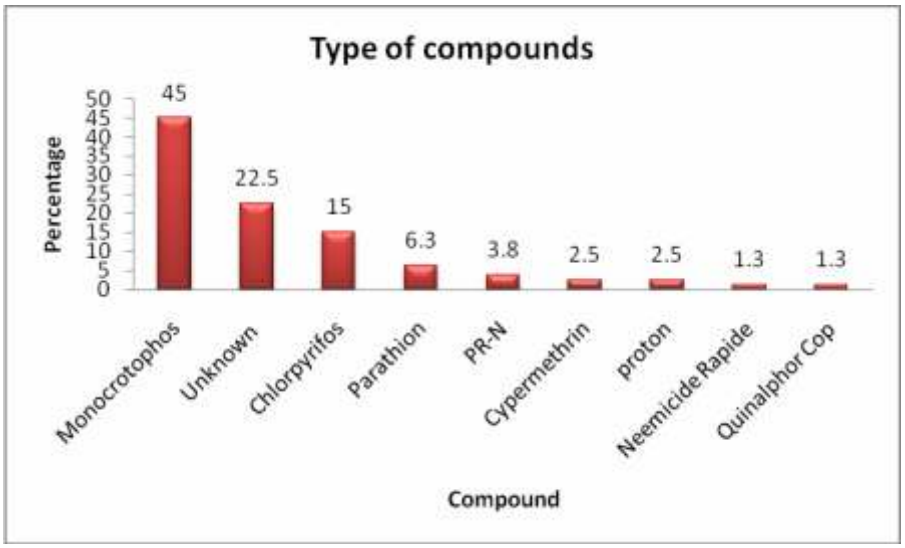


Fig. 2: Distribution of Compound

Table 3: Comparison of Plasma Cholinesterase and Serum Amylase Level by Days

Day	Plasma Cholinesterase (N=80)	Serum Amylase (N=80)
	Mean ± SD U/L	Mean ± SD U/L
Day 1	3369.0 ± 3193.2 (80-9800)	196.6 ± 143.4 (27-714)
Day 3	3781.3 ± 3216.8 (111-11354)	159.3± 113.9 (34-638)
Day 5	5271.6 ± 3322.7 (603-11857)	100.6 ± 069.0 (34-530)
ANOVA p Value	<0.001	<0.001

Discussion:

In this study male: female ratio is 1.3:1 due to male preponderance. A study conducted by Gupta *et al* on 413 patients has found male: female ratio of 1.9:1 [11]. Incidence of OP has been common among 21-30 years. This is the most critical period, when one is likely to face various problems that may lead to psychological stress so a person may take drastic steps to end his life, consuming available poisons. Similar results have been in studies conducted by

Gupta *et al* [139/413 (33.6%) in age group 21 to 30], Dayanand Raddi *et al* [144/320 (45.0%) in age group of 21 to 30], Hundekari *et al* [58/92

(63.0%) cases in age group of 21 to 30]. A study conducted by Gupta *et al* out of 413 patients, 139 cases was in the age group 21-30 years [11, 3, 12]. Farmers are more proned for OP poisoning which might be due to easy availability and accessibility of pesticides among them. Vomiting is most common symptom because of the practice of induced emesis by giving salt water in our area because of lack of knowledge. Suicidal poisoning has been commonest mode of poisoning (93.4%). Rapid urbanization, socio-economic factors mainly contribute to frustration and depression in the people. The persons who are not able to cope up with the stressful situations are the major victims of suicidal poisoning. Choice of OP compound for suicide is mainly due to cheap and easy availability. Similar result have been seen in a studies conducted by Dayanand Raddi *et al* [312/320 (97.5%)] [3], Hundekari *et al* [143/150 (95.3%)] [12], Kar *et al* [64/65 (95.2%)] [13].

Monocrotophos is the commonly used pesticide in the paddy fields and it's easily available in the market. Monocrotophos 124 (30.17%) is the most common compound in a study conducted by Gupta *et al* followed by methyl parathion 117 (28.44%), and quinolphos 78 (18.96%) [11].

Plasma cholinesterase inhibition correlates well with the clinical severity in case of OP poisoning. Our findings of reduction of plasma cholinesterase on the day of admission and severity of OP poisoning are consistent with Wadia *et al*, who has shown good correlation between plasma cholinesterase level and severity of poisoning [5]. Plasma cholinesterase has shown a trend of increase in activity with treatment of atropine and pralidoxime (PAM) during the course of hospital stay.

Mean serum amylase at admission has been 196.6 units in this study which has decreased to 100.6 units on 5th day. This shows that in OP poisoning there is elevation of serum amylase level according to the degree of cholinergic stimulation. Our findings correlate with Lee *et al* who have demonstrated hyperamylasemia is frequent in severe OP poisoning and finding of hyperamylasemia is closely related to clinical severity [14]. There has been progressive decrease in the mean serum amylase levels from admission to 5th day. The present study also shows that greater the serum amylase at admission, higher have been the rate of complications and prolongation in the hospital stay. In a study conducted in Japan by Sumiya *et al*, an increase in plasma amylase levels above the normal range have been found in 50% of the patients who developed respiratory failure. The study has found a positive correlation with amylase levels with respiratory failure in organophosphorus poisoning [15]. Bhardwaj *et al* have found that serum amylase is elevated in 47% of patients with organophosphorus poisoning [16]. Sahin *et al* have conducted a study and acute pancreatitis is observed in 12 (12.76%) patients [17]. A study is conducted by Kumar *et al* in 31 patients. Serum cholinesterase has been estimated at admission, at 48, 72 and 120 hours. Based on serum cholinesterase they have divided the poisoning as mild (<10% reduction), moderate (10-50% reduction) and severe (>50% reduction). The study has shown significant increase in serial serum cholinesterase levels were correlating with better clinical outcome as evidenced by improvement with mechanical ventilation and survival rates in acute OP poisoning [18]. Patil *et al* have conducted a study in 82 patients. Serum cholinesterase has been estimated on admission and has divided into three groups: mild (>4500 IU/L), moderate (4500-2500 IU/L), severe (<2500 IU/L). Study has shown low serum cholinesterase level on admission is a strong predictor of mortality and requires intensive care treatment and higher dose of atropine [19]. In our study, we have observed that patients with low serum

cholinesterase levels on admission stayed for longer time in hospital and required ICU care and those with low serum cholinesterase levels required significantly higher dose of atropine compared to patients with high cholinesterase levels. In a study conducted by Pendkar *et al*, ninety patients based on signs and symptoms have been graded in three groups. Serum amylase has been considerably high in patients with grade 2 and 3. Acute pancreatitis is observed in three patients [20]. Patil *et al* have graded 60 patients into mild, moderate and severe groups based on Peradeniya OP poisoning scale. The study has shown that serum amylase has been elevated in 18 (30%) patients and 12 (66.7%) patients with elevated serum amylase levels have required ventilator support [21]. A study has been conducted by Sumathi *et al* in 53 patients based on serum cholinesterase activity at the time of admission have formed three groups: group 1 having 20-50% of serum cholinesterase activity, group 2 having 10-20% of serum cholinesterase activity and group 3 <10% of serum cholinesterase activity. Study has indicated that mean amylase levels are elevated in patients with respiratory support and serum amylase could predict ventilator support in OP poisoning. Group 3 patients have increased amylase level and have shown significant negative correlation with serum cholinesterase levels [22]. Our study has shown there is a significant negative correlation with serum cholinesterase levels and our study found positive correlation with respiratory failure with increase mean amylase levels.

Conclusion:

With this we conclude that OP poisoning is associated with hyperamylasemia.

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