

Study of serum cholinesterase, CPK and LDH as prognostic biomarkers in Organophosphorus Poisoning

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Abstract

Introduction: Organophosphorus insecticides are arguably one of the commonest causes of morbidity and mortality due to poisoning worldwide, especially in developing countries like India due to its easy availability. This study was conducted to estimate the levels of Serum Cholinesterase, Serum Creatinine Phosphokinase & Serum Lactate Dehydrogenase on admission for correlation with severity of organophosphate poisoning and to reevaluate their levels on Day 4 and Day 8 and correlate them with the complications and final outcome of the poisoning. **Material and methods:** This is a single centered cross sectional study over a period of one year. 100 patients of OP poisoning were selected and their clinical severity was categorized according to Peradeniya organophosphorus poisoning (POP) scale. Level of serum Cholinesterase, serum CPK, and serum LDH were measured at admission and on Days 4 and 8. **Results:** Serum Cholinesterase and CPK levels strongly correlated with clinical severity. The comparative values of serum Cholinesterase and serum LDH amongst survivors and non survivors during the course of the study was not statistically significant. The comparative values of serum Creatinine Phosphokinase between survivors and non survivors showed a statistically significant difference. **Conclusion:** Serum Cholinesterase serves as a diagnostic parameter for organophosphorus poisoning and correlates with the severity but it cannot be used as a prognostic biomarker. Serum Creatinine Phosphokinase shows a strong degree of positive correlation with the severity of poisoning and can be used as a predictor of outcome in organophosphorus poisoning.

Key words: Cholinesterase, Creatinine Phosphokinase (CPK), Lactate dehydrogenase (LDH), Organophosphorus (OP), Peradeniya organophosphorus poisoning (POP) scale.

Introduction

The World Health Organization (WHO) estimates that globally three million intentional or unintentional pesticide poisoning episodes occur annually and out of these a minimum of 300, 000 die [1]. It seems these data represent only the tip of ice berg as most cases are not reported from developing nations.

Organophosphorus insecticides are arguably one of the commonest causes of morbidity and mortality due to poisoning worldwide, especially in developing countries like India due to its easy availability. The morbidity and mortality outcome depends on the time lag between exposure and the onset of management. So, it is cardinal to recognize the entire spectrum of the symptoms. Organophosphorus (OP) compounds inhibit acetyl cholinesterase and butyryl cholinesterase enzymes resulting in over stimulation at

cholinergic synapses [2]. Symptoms are classified into muscarinic, nicotinic and central depending on the actions over the respective receptors. Muscarinic features include excessive salivation, lacrimation, urination, diarrhea, gastrointestinal cramps, emesis, blurred vision, miosis, bradycardia, and wheezing. Nicotinic features include fasciculation, paresis or paralysis, hypertension and tachycardia. Three types of neuro muscular paralysis are noticed. Type I is due to continued depolarization at neuro-muscular junction, type II due to intermediate syndrome and type III due to delayed polyneuropathy. Central features include anxiety, confusion, seizures, psychosis and ataxia [3, 4].

The quest for newer biomarkers in relation to OP compound poisoning started quite a long time back. OP labeled albumin in plasma, blood β -glucuronidase and paraxonase status have been suggested by some researchers to be very reliable marker for both diagnosis and prognosis. But all these assays are dependent on

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specialized laboratories and costly. Many studies have been undertaken to investigate the diagnostic and prognostic value of serum cholinesterase levels in organophosphorus (OP) poisoning and their relationship with the neurological syndromes (intermediate syndrome and delayed polyneuropathy) [5,6].

Most of the studies have shown that though serum cholinesterase can be used as a diagnostic marker, its role is minimal as a prognostic marker [7, 8]. So cheap and easily measurable biomarkers having prognostic value is the need of the hour [9- 12].

It was proved beyond doubt that rhabdomyonecrosis occur in animals after experimental poisoning by OP compounds [13]. After the publication of this study in 1993, there have been many human studies which show elevations in Serum CPK and Serum LDH levels following acute OP poisoning as a result of muscle injury. There is also biopsy proven evidence of muscle fiber necrosis in humans [14].

A number of recent studies have been carried out using parameters like serum CPK, serum LDH, serum amylase, liver enzymes and urine creatinine levels as new markers and their correlation with the severity and prognosis of OP poisoning [9- 12]. This study is conducted to observe if the levels of Serum Cholinesterase, Serum CPK and Serum LDH can be used to assess the severity of OP poisoning and to serve as prognostic biomarkers of OP poisoning.

Material & methods

This is a cross sectional study which was conducted in a tertiary health care centre over a period of one year. Detailed examination was conducted with a preset questionnaire.

Inclusion criteria

1. Age more than 15 years and
2. Who has consumed OP compounds and
3. Who have arrived to the hospital within 6hr. of consumption.

Results

Out of the total 100 cases, females outnumbered the males (57 Vs 43). Looking at the demography, majority of patients were from low socioeconomic strata and were young. Forty out of 100 patients were in the age group between 15 and 24 years of age. Fixed dose preparation of Chlorpyrifos and Cypermethrin was the most common type of compound used for poisoning (28 out of 100 cases) followed by Phorate (23 cases) and Chlorpyrifos (15 cases). Other OP compounds encountered were Acephate, Malathion, Parathion, Propenophos, Quinalophos, and Triazophos. Salivation and vomiting were the most common symptoms (in 77 and 75 cases respectively) followed by abdominal pain/loose motion in 24 cases. Bradycardia was the most common symptom followed by constricted pupil and tachypnoea (in 69, 64 and 56 cases respectively).

Exclusion Criteria

1. Patients with other co-existing illness (myopathy, chronic renal disease, epilepsy, myocardial infarction, myocarditis, autoimmune diseases or malignancy).
2. Patients who had trauma or received intramuscular (I/M) injection and cardiopulmonary resuscitation recently.
3. Patients who are on prior medications like statins, fibrates, aspirin, anticoagulants, frusemide, and dexamethasone or alcoholic.

After carefully considering the inclusion and exclusion criteria, 100 cases were included in the study. Relevant parameters were compared using SPSS 19 software. Student's t test was used for nominal variables and chi square test was used for categorical variables. Patient characteristics and outcome of interest was calculated with 95% confidence limits. The probability of <0.05 is considered to be significant.

Type of poisoning was ascertained by objectively looking at the packet/ container. Clinical severity of poisoning was categorized according to the Peradeniya Organophosphorus Poisoning (POP) Scale proposed by Senanayake et al in 1993 [15]. It identifies clinical parameters representing the muscarinic, nicotinic and central actions of OP compounds which can grade severity without laboratory investigations. It also does not rely on patient cooperation and hence can be applied to all groups of patients without the need of their cooperation (Table 1).

Samples were collected on the 1st, 4th and 8th day of admission with a single prick from a peripheral vein with all available aseptic procedures. Serum cholinesterase, CPK and LDH levels were measured by spectrophotometric analysis. Then their levels were studied with the POP Scale severity. The value of serum Cholinesterase 4620 to 11500 U/L in males and 3930 to 10800 U/L in females was taken as normal. For CPK, the normal value in male was 55-170 U/L and in female 30-135 U/L. For serum LDH, 313-618 U/L was taken as normal values for both male and female.

Table 1: POP scale and correlation with mean serum cholinesterase, CPK and LDH at the time of admission

POP score	No. of patients	Serum cholinesterase Mean±SD	Serum CPK Mean±SD	Serum LDH Mean±SD
0 – 3	29	2006.75±1554.7	449.65±325.4	329.0±125.47
4 – 7	45	1389.27±1544.0	768.2±485.4	480.31±310.05
8 – 11	26	838.70±699.59	1324.74±141.6	497.88±321.87

According to the severity of poisoning as per the POP Scale, 29 patients (23 female and 6 male) had mild poisoning, 45 had moderate poisoning (22 females and 23 males) and 26 (12 females and 14 males) had severe poisoning. Patients having mild, moderate and severe poisoning were grouped and it was seen that with increase in the severity of poisoning, there is a decrease in the mean value of S. Cholinesterase level and increase in the mean value of S. CPK and S.LDH (Table 1).

There was negative correlation (Pearson coefficient $r = -0.267$) between severity of poisoning and serum Cholinesterase levels and the correlation was statistically significant ($p = 0.001$). The correlation between severity of poisoning and serum CPK showed a high degree of positive correlation ($r = 0.625$) and the correlation was also statistically significant ($p = 0.001$). The correlation between serum LDH and severity of poisoning was also positive with a value of $r = 0.291$, but not statistically significant.

Among the de novo in hospital complications respiratory acidosis was most common and was found in 55% cases. 15 patients (9 male and 6 female) developed the dreaded intermediate syndrome. Other documented complications were seizure (7 male and 3 female) and coma (4 male and 2 female). The hospital mortality rate was 22%. None of the patients died from the mild poisoning group. Five patients (3 male and 2 female) having moderate poisoning and 17 patients (10 male and 7 female) with severe poisoning succumbed during the treatment.

Table 2: Comparison of mean serum cholinesterase values in survivors and non survivors on day 1, 4 and 8

Day	Survivors cholinesterase Mean ±SD	Non survivors cholinesterase Mean ±SD	Difference	P value
Day 1	1593.23±1539.2	802.80±652.97	790.43	0.0225
Day 4	1935.33±1497.8	1189.03±1023.06	746.33	0.0707
Day 8	2264.74±1413.8	1745.00±1662.6	519.74	0.9870

There was statistically significant rise in the initial and final mean values of serum Cholinesterase in cases of mild poisoning but the difference was not statistically significant in moderate or severe poisoning. Serum Cholinesterase values show a progressively increasing value in both survivors and non survivors (Table-2).

Table 3: Comparison of mean serum CPK values in survivors and non survivors on day 1, 4 and 8

Day	Survivors CPK Mean ±SD	Non survivors CPK Mean (±SD)	Difference	P value
Day 1	698.58±486.48)	1277.81±645.23)	579.23	0.002
Day 4	595.37±587.32)	1180.54±729.94)	585.17	0.0017
Day 8	332.527±276.97)	986.5±635.34)	654.0	0.710

However, the serum CPK levels showed a statistically significant difference between the initial and final values in mild, moderate and severe poisoning. In comparative studies between survivors and non survivors, the difference between mean S. CPK levels in survivors and non survivors on Days 1 and 4 were statistically significant in mild and moderately severe cases (p values of 0.002 and 0.0017 respectively). In survivors, the levels of serum CPK showed a progressively decreasing trend while in non survivors; the CPK value remained persistently elevated above 900. The study also showed that if the Serum CPK value remains persistently above 900 IU /L throughout the course of the disease, it is associated with the development of complications and with higher mortality rate (Table-3).

The serum LDH levels did not show statistically significant difference between the initial and final values in mild, moderate or severe poisoning. In comparative studies between survivors and non survivors, the difference between mean serum LDH levels in survivors showed a progressively decreasing trend but the difference in values between survivors and non survivors was not statistically significant.

Discussion

Looking at the demographic profile in our study it is no wonder that most of the patients are from low socioeconomic status and young. This young age presentation is probably due to the fact that they are more emotionally liable and jobless which make them more vulnerable to contemplate drastic steps like suicide. Female has outnumbered males in our study emphasizing the fact of more labile emotions in them. But looking closely it is evident that maximum cases among females are mild to moderate (45 out of 57 cases) where as in males maximum cases were moderate to severe (34 out of 43 cases). This is very unfortunate to know that so many young people are dying consuming this easily accessible notorious insecticide. So there must be some constraints for its availability in the market.

Yves Vanneste & Dominique Lisson had shown that rhabdomyonecrosis occurs after organophosphate poisoning and was accompanied by a concurrent increase in serum total creatine phosphokinase activity and urinary creatine excretion rate [13]. In a study performed by M. John, A. Oommen, A. Zachariah in Christian Medical College Vellore, muscle injury was seen in all patients beginning at admission, peaking over the first 5 days and then declining over the next 5 days. Temporal profiles of muscle isoenzyme of CPK showed significantly greater muscle injury in those patients with greater severity of poisoning at admission, those who developed intermediate syndrome and in patients with longer duration intermediate syndrome. The mechanism of acetylcholinesterase inhibitor-induced myopathy has been described as an “agonist-induced myopathy” with per-junctional sarcoplasmic swelling and organelle damage, Z band destruction and focal contractures. In our study also we observed that serum CPK level is elevated even in the absence of intermediate syndrome, provided the patient is severely poisoned, presumably due to muscle fiber necrosis. There is a strong positive correlation between the clinical severity of poisoning and the initial levels of serum CPK. If there is ongoing injury to the muscle due to development of complications, the CPK level continues to be elevated. In other words high and persistent serum CPK can be taken as a marker of impending complications.

In our study, there was negative correlation (Pearson coefficient $r = -0.267$) between severity of poisoning and serum Cholinesterase levels and the correlation was statistically significant ($p = 0.001$). This means that serum Cholinesterase levels can be a reliable biomarker for the diagnosis. But the comparative values of serum Cholinesterase amongst survivors and non survivors during the course of the study were not statistically significant suggesting that it is not a reliable indicator for prognosis. So it can only be used in diagnosing suspected OP compound poisoning at present without any prognostic significance.

The comparative values of serum CPK between survivors and non survivors during the course of the study showed a statistically significant difference suggesting that it can be used as a measure of prognostic significance. To be more specific if the Serum CPK value remains persistently above 1000 IU /L throughout the course of the disease, it is associated with higher rate of complications and mortality. The difference between mean serum LDH levels in survivors and non survivors was statistically not significant suggesting that that it is not a reliable indicator for prognosis and since LDH is not specific to muscle so its elevation does not necessarily point a diagnosis.

Conclusion

It may be concluded from the study that serum Cholinesterase serves as a diagnostic parameter for organophosphorus poisoning and correlates with the severity but it cannot be used as a prognostic biomarker.

Serum Creatinine Phosphokinase shows a strong degree of positive correlation with the severity of poisoning and can be used as a predictor of outcome in organophosphorus poisoning. Serum Lactate Dehydrogenase can neither be used as a marker of severity nor as a prognostic biomarker. The study has its

own limitations as it was carried out involving a relatively small number of subjects with limited parameters. Further studies involving different parameters with a larger number of patients may be carried out to validate the accuracy of our study claiming that serum CPK can be used for the predictor outcome in OP poisoning.

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