CLINICAL UTILITY OF CREATINE KINASE AND LACTATE DEHYDROGENASE AS SEVERITY MARKERS OF ORGANOPHOSPHOROUS COMPOUND POISONING

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ABSTRACT
Organophosphorus poisoning is a major public health problem in developing countries. In India, rate of suicidal poisoning with OPC ranges from 10.30 to 43.8%. Aim and objective: 1. To study the changes in serum levels of LDH and CK in patients with organophosphorus compound poisoning. 2. To correlate the severity of the poisoning with the levels of CK & LDH. Materials and Methods: In this prospective study we included 60 patients - 32 males and 28 females. The mean age of patients was 31.5 years. Based on Peradeniya Organophosphorus Poisoning (POP) score patients were divided into (42.5%) mild, (37%) moderate, (20%) severe cases. Results: A significantly increased serum CK, LDH levels in OPC poisoning cases than controls. The mean levels of CK and LDH is higher in severe POP score patients. The mean levels of CK and LDH in higher in severe POP score patients (CK =312.2±53.1 IU/L, LDH=690.1±42.5 IU/L) than moderate POP score patients (CK =291.4±75.7 IU/L, LDH=466.8±67.3 IU/L). The mean levels of CK and LDH higher in moderate POP score patients (CK =291.4±75.7 IU/L, LDH=466.8±67.3 IU/L) than mild POP score patients (CK =157.8±48.8 IU/L, LDH=266.7±29.5IU/L). conclusion: Early assessment the severity marker is very important reduce the mortality. CK, LDH levels can be an efficient biomarker and can predict the prognosis OPC poisoning.

KEYWORDS: Organophosphorus Compound (OPC), lactate dehydrogenous (LDH), creatine kinase (CK)
Peradeniya organophosphorus poisoning (POP).

INTRODUCTION
Organophosphorus compound (OPC) poisoning is the most common insecticide poisoning worldwide. According to World Health organization (WHO), two million people are hospitalized for suicide attempts with OPCs compounds.[1,2] In India, the incidence of OPC poisoning was 1.26 lakhs, as reported by Ravi et al 2007. The commonly used OP compounds are malathion, parathion, dichlorvos, nerve gases, ophthalmic agents like echothiopate, herbicides etc.[3] In India OP compounds are easily available and abundantly used for farming. Since it is cheap, it is a source for both intentional and unintentional suicidal poisonings.[4]

organophosphorous compounds inhibit carboxylic esterase, acetylcholinesterase enzymes found in erythrocyte, neurons, skeletal muscle, liver, heart. most of clinical manifestation attributed to inhibition of this enzyme.[5]

Estimation of erythrocyte cholinesterase is used for lab diagnosis of OP poisoning, but they are costly and have interpersonal variability, hence this study was designed to assess if CPK and LDH can be used as easily available biomarkers to stratify OP poisoning severity.[6,7]

PATHOPHYSIOLOGY
OP compounds are irreversible inhibitors of carboxylic ester hydrolases which include acetyl cholinesterase, erythrocyte cholinesterase and nonspecific proteases. The primary toxicity is due to sustained stimulation of muscarinic and nicotinic cholinergic receptors (figure1) by accumulation of acetylcholine in the central and autonomic systems as well as skeletal neuromuscular junctions. OP insecticides increase reactive oxygen species which results in oxidative stress, lipid peroxidation, DNA damage and cell death. Activation of proteases, caspases and phospholipases will cause the muscle damage. Rhabdomyolysis will release the muscle enzymes like Creatine Kinase and Lactate dehydrogenase.[8,9,10](Figure1).
Clinical findings
Patients with OP compounds ingestion usually presents with miosis, bradycardia, increased GI motility, emesis, lacrimation, salivation, photophobia due to inhibition of muscarinic and nicotinic receptors. Complications include acidosis, respiratory failure, acute renal failure, arhythmias and coma. According to Peradeniya organophosphorus poisoning scale, the severity of poisoning is classified into three categories based on clinical parameters: 0–3, mild poisoning; 4–7, moderate poisoning; 8–11, severe poisoning groups (Table 1).

Laboratory evidence of poisoning is measurement of acetyl cholinesterase activity which is decreased. Because of inter-individual variability, false negative values and high cost, they are not routinely measured. The emerging options which on supplement or replace acetyl cholinesterase activity is measurement of Creatinine kinase and Lactate Dehydrogenase levels in serum. These are cheaper and easy to quantify even in tertiary care hospitals.

AIMS AND OBJECTIVES
1. To study Lactate Dehydrogenase and Creatinine Kinase-MB in patients with Organophosphorus compound poisoning.
2. To correlate the severity of the OPC poisoning with the levels of the enzymes.
3. To assess the levels of the enzymes as alternate prognostic marker in OPC poisoning.

MATERIALS AND METHODS
Study design: This prospective observational study was done during the period from February 2013 to January 2014.

Study centre
This study was conducted at Government Villupuram Medical College and Hospital, Villupuram, Tamil Nadu, India. This is a 500 bedded, tertiary care level teaching institute located in a rural district hospital of southern India. The study was done by the department of biochemistry and internal medicine in unison.

Subject selection
The study was done after getting approval from the Institutional ethical committee. A total of 60 patients were enrolled into the study, patient enrollment was done based on inclusion and exclusion criteria outlined below:

Inclusion criteria
• Hospitalized, adult patients with OPC poisoning, who presented within 6 hrs of the ingestion of OPC compound.

Exclusion criteria
• Adult patients who presented later than 6 hrs.
• Patients in whom the ingestion could not be identified.
• Patients with severe co morbid illnesses like chronic kidney disease, heart failure, diabetes etc.

METHODODOLOGY
The sample included 60 patients and 60 age and sex matched healthy controls.

Then study samples were grouped into mild, moderate and severe cases based on Peradeniya Organophosphorus Poisoning scale (POP) Table 1.

Biochemical analysis
5 ml of venous sample was collected from study participants. The blood samples were centrifuged 3000rpm for 10 minutes and serum collected. Measurement of serum acetyl cholinesterase, Creatine Kinase and Lactate dehydrogenase were done schumann (2002) in fully automated clinical chemistry analyzer (Beckman Coulter). The findings were computed periodically.

Determination of Creatine Kinase Assay
The Creatine Kinase Activity was assayed by immunoinhibition method Soe(2005). 1 ml of reconstituted reagent was mixed with 20µl of serum. Creatine Kinase, if present in the serum releases the phosphate group from creatine phosphate. The phosphate group combines with glucose to form by the enzyme hexokinase. Glucose-6-Phosphate is oxidized to produce NADPH. The increasing absorbance of NADPH is monitored at 340 nm and is directly proportional to CK activity. Increasing optical density was measured 4 times at one minute interval. The average absorbance difference per minute was calculated. The Abs/min was multiplied by the factor 8095 to get CK activity in IU/L.

Determination of Lactate Dehydrogenase assay
The Lactate Dehydrogenase activity is estimated by kinetic method. 1ml of reconstituted reagent was mixed with 20µl of serum. The pyruvate in the reagent is catalyzed by LDH to form lactate and NAD. Decrease in absorbance due to oxidation of NADH is monitored at 340nm & is directly proportional to LDH activity. Decreasing optical density was measured 4 times at one minute interval. The average absorbance difference per minute was calculated. The Abs/min was multiplied by the factor 8199 to get lactate dehydrogenase activity in IU/L.

RESULTS
Out of the 60 patients enrolled in the study, 38 were men and 22 were women. They were aged from 20 to 40 years. Most of the patients were from rural background, predominantly from lower socioeconomic status according to Modified Kuppusamy scale. They were mostly farmers, housewives and daily wage laborers. Results were analyzed with SPSS software. Mean values, standard deviation, student’s t test were used for assessment of statistical significance.

According to Peradeniya POP scale, 25 out of 60 (42.5%), 22 out of 60 (37%), 13 out of 60 (20.5%) were considered as mild, moderate, severe cases of poisoning respectively (Figure 2). CK and LDH levels are increased in cases (CK=239 ± 92.6 IU/L, LDH = 427.5±168.4 IU/L) when compared with age and sex matched controls (CK=20.6 ± 2.4 IU/L, LDH = 207.5± 57.1 IU/L). It shows statistical significance p value=0.000. (Table 2).

Table 3. Shows that mean levels of CK and LDH are higher in severe POP score patients (CK =312.2±53.1 IU/L, LDH=690.1±42.5 IU/L) than moderate POP score patients (CK =291.4±75.7 IU/L, LDH=466.8±67.3 IU/L). The mean levels of CK and LDH are higher in mild POP score patients (CK =291.4±75.7 IU/L, LDH=466.8±67.3 IU/L) than moderate POP score patients (CK =175.8±48.8 IU/L,LDH=266.7 ±29.5IU/L). (Figure 3).

Table 2 Comparison of CK, LDH levels between cases and controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK (IU/L) Mean ± SD</td>
<td>239.0 ± 92.6</td>
<td>20.6 ± 2.4</td>
<td>0.000</td>
</tr>
<tr>
<td>LDH (IU/L) Mean ± SD</td>
<td>427.5 ± 168.4</td>
<td>207.5 ± 57.1</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Figure.2 Peradeniya OPC Scale
Figure.3 CK,LDH levels between mild, moderate and severe patients.
Table 3. Comparison of mean±SD levels of CK,LDH between mild, moderate and severe patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK (IU/L) Mean ± SD</td>
<td>157.8 ±48.8</td>
<td>291.4 ±75.7</td>
<td>312.2 ±53.1</td>
<td>0.000</td>
</tr>
<tr>
<td>LDH(IU/L) Mean ± SD</td>
<td>266.7 ±29.5</td>
<td>466.8 ±67.3</td>
<td>690.1 ±42.5</td>
<td>0.000</td>
</tr>
</tbody>
</table>

DISCUSSION

OP poisoning is a major health problem worldwide and one of the commonest causes for morbidity and mortality, particularly in developing countries. The prognosis depends on time lag between exposure and the onset of management. With increase in use of this compound for industrial and agricultural purposes and due to easy accessibility and low cost, they are becoming major source of health hazard. Early identification, diagnosis, appropriate management are vital to reduce the mortality.  

Most of the cases were young people between 20 and 40 years as supported by the study of khan RA et al. Pyar (2012)[23], vander Hoek et al. Most common symptoms noted in the study were miosis, salivation, anxiety, lacrimation, bronchospasm and restlessness. Types of paralysis are at neuromuscular junction (type I), intermediate syndrome (type II) and delayed neuropathy (type III) which leads to muscle fiber necrosis [24].

A study conducted by Sharma et al showed that muscle enzymes are increased maximally in severe poisoning case.

The results also confirm the findings of Bhattacharya et al showing that there is correlation between CK values and POP scale, Serum EChE levels, arterial pH values and total dose of atropine. The injury of the skeletal muscle leaks CK into the blood and urine. Serum CPK and LDH levels remain the best biomarker for detecting and monitoring skeletal muscle damage and diseases which was confirmed by Perreault et al.[21]

Acidosis is a common complication in OP poisoning and it influences the outcome of poisoning. Those with acidosis have higher mortality rate compared to those without acidosis. Liu et al correlated CPK values with degree of acidosis and severity of OPC poisoning.

Sahjian and Frakes stated that if there is ongoing injury to the muscle due to development of complications, the CPK Level continues to be elevated since half-life is about 1.5 days. It normalizes within 5-6 days of a single insult to the muscle.

Counselman et al stated that muscle enzymes peak within 24 to 48 hours of the onset of muscle injury or rhabdomyolysis and decline at a relatively constant rate of 39% of the previous day’s value.

In our study, we found that CPK, LDH levels are elevated in acute poisonings cases.

CONCLUSION

Pesticides are major chemical agents which pose great health threat particularly to young people, farm workers and depressed individuals. Hence rapid diagnosis, early and effective treatments are essential. Serum CK, LDH levels can be an efficient biomarker in case of acute OP poisoning. Serial measurement of these enzymes can predict the prognosis OPC poisoning.

LIMITATIONS

These parameters cannot be specific markers to the OP compound poisoning. Even it cannot be a specific marker, can predict the prognosis OPC poisoning.

ACKNOWLEDGEMENT

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REFERENCES


