Alternate Biochemical Markers in Organophosphate Poisoning

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ABSTRACT

In India, organophosphates are the most widely used pesticides for suicide by poisoning. Early recognition of the diagnosis and its severity will help in achieving a better outcome. In poisoning by organophosphorus compounds, serum acetylcholinesterase (AChE) and pseudocholinesterase are currently widely accepted as biochemical markers for estimating the severity. A wide array of alternate, cheap, and easily available markers are explored in this review and using a combination of these markers may be better in terms of early identification of severe poisoning. In peripheral centers without access to costly investigations, these cheap markers may help in guiding an early referral to higher centers for severely poisoned patients. A comprehensive study comparing all these different markers has not been done so far, thereby emphasizing the need for the same. This review identified various new, cheaper, and easily available biochemical markers as having the potential to act as surrogates for assessing the severity of organophosphate poisoning, and there is a scope for future studies to understand its utility.

INTRODUCTION

Poisoning by organophosphates (OP) is recognized as a serious medical problem globally, especially in India, where the majority of the population relies on agriculture for their livelihood.

GLOBAL SITUATION

As per the latest statistics published by the WHO in 2019, around 8 lakh deaths occur each year from suicide, amounting to 1 death every 40 seconds, and it is estimated that deliberate self-harm using pesticides amount to 20% of the total deaths. Suicide is a global phenomenon. A total of 77% of the suicides happened in “low and middle-income countries” in the year 2019.1

IN INDIA

A total of 318 pesticides have been registered in India as of October 2019. According to the WHO toxicity criteria, 18 belong to class 1a or class 1b (extremely or highly hazardous, respectively) pesticides.2 In a nationally representative mortality survey published in 2012, about 3% of deaths ≥15 years were by suicide. About half of these suicides were from poisoning, most of which was pesticide.3 Compared to high-income countries, suicide in India is more due to the consumption of pesticides, related to poverty, and also to psychiatric illness to a lesser extent.4 All these dangerous pesticides are used in agricultural work and hence easily available for poisoning. A reduction in occupational and suicidal poisoning by pesticides can be achieved by a national ban on these pesticides across India. As per the latest statistics published in 2019,2 the highest number of suicides in 2019 occurred in Maharashtra, with Kerala being in the sixth place (Fig. 1). Kerala came in third place after Sikkim and Chhattisgarh with 24.3 per 1 lakh population in 2019 (Fig. 2) when suicide rates were calculated.

When the means of suicide were analyzed, poisoning (26.7%) came in second place only to hanging (51.5%) (Table 1).

The most common poisons used for suicide in India from 1999 to 2018 were published in 20216 in a systematic review. It concluded that, after the government regulatory changes in 2001, organophosphates replaced aluminium phosphide as the key lethal poison in India. Medication overdose, hair dye, and plant poisoning caused only a few deaths. Aluminium phosphide contributed to fatality mainly in North India, but deaths due to OP poisoning occurred all over India. In the last 10 years, paraquat poisoning has been recognized as an important health issue. Pesticide poisoning deaths are still very common, emphasizing the need for regulatory interventions to reduce the burden of pesticide poisoning deaths in India.

OVERVIEW OF ANTICHOLINESTERASE POISONING

Pesticides include insecticides, herbicides, and rodenticides. Organophosphates, the focus of this article, belong to the class of insecticides. Other classes of insecticides include carbamates, organochlorines, pyrethrins/pyrethroids, neonicotinoids, and nereistoxin analogs. Organophosphates are irreversible anticholinesterases; that is, they are irreversible inhibitors of the cholinesterase enzyme, whereas carbamates are reversible inhibitors.

The neurotransmitter acetylcholine facilitates communication between a neuron and a target cell (a gland, a muscle cell, or another neuron). When stimulated, acetylcholine is released into the synapse. It binds to receptors on the target cell (a gland, muscle, or neuron). This results in muscle fiber activation if the target cell is a muscle. It can also cause variations in heart rate, glandular secretions or interneuronal communication in the brain or autonomic ganglia. Cholinergic pathways are present ubiquitously in the human body in every organ system. Compounds inhibiting the cholinesterase enzyme can inhibit both sections of the human nervous system [the peripheral nervous system (PNS) and the central nervous system (CNS)], as they both have cholinergic neurons (Fig. 3). The PNS consists of acetylcholine-releasing neurons at the neuromuscular junctions and in the autonomic nervous system (glands, ganglia, smooth muscle, and cardiac muscle). The CNS is the other section, and this section also contains cholinergic neurons. They are present in both the brain and also in the spinal cord in the intermediolateral cell column, which regulates autonomic functions.

The cholinergic receptor distribution in the CNS and the PNS is not the same. For example, the striatum, hippocampus, and cerebral cortex are rich in cholinergic neurons, while other regions like the cerebellum have less of the same. Muscarinic and nicotinic receptors are the two types of cholinergic receptors. Both have many subtypes as well. There are differences in the way they are distributed as well, making their effects even more complex. The synapses of neurons releasing acetylcholine contain an enzyme called acetylcholinesterase (AChE), mainly at

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the neuromuscular and other neuroeffector junctions. It degrades acetylcholine and thereby ends its action in the synapses. Therefore, inhibition of this enzyme can cause acetylcholine to accumulate and hence prolong its action. In the PNS, the accumulation of acetylcholine at nerve endings can produce cholinergic symptoms. This can include abdominal cramps (due to contraction of the smooth muscle around the gut), sweating, muscle spasms and twitching, and even flaccid paralysis at higher concentrations. There may also be effects on behavior and on, memory, and learning in the case of chronic poisoning. Thus, we see that AChE inhibition can cause a lot of adverse effects, and depending on the degree of exposure and half-life of the compound, they may be fatal. These symptoms can be due to action on the CNS or the PNS, as explained already. The compounds vary in their ability to enter the CNS or PNS. This is because these two sections differ in their pharmacokinetic properties. These differences may be due to differences in chemicals, or they may be organism-specific. It can also depend on the pharmacodynamic properties of the drug/poison and the type of enzyme it interacts with. Even though butyrylcholinesterase (BuChE)/pseudocholinesterase is similar to AChE in structure, a different gene is responsible for its production. BuChE is produced mainly in the liver. It is also present in plasma and some other tissues. It is usually differentiated from AChE by the fact that it hydrolyses acetylcholine much slower and also by histochemical techniques. Also, the binding affinity of each anticholinesterase to these two enzymes is different. Both AChE and BuChE are present during the nervous system development, but their relative proportions keep changing with location and time. Even though BuChE was found to have no function in the nervous system, in plasma, it was seen to be catalyzing the breakdown of certain ingested plant esters (like cocaine) as well as synthetic local anesthetics and paralytic agents such as succinylcholine. Similarly, erythrocyte AChE also has no known function. These enzymes are considered surrogate measures of cholinesterase activity. Red blood cells contain AChE but no BuChE, while plasma has both, even though their ratio varies widely among humans and animals. Human plasma contains mainly BuChE, while dogs and rats have both AChE and BuChE in considerable amounts in their plasma. It is still yet to be found out if BuChE helps in the development of the nervous system and, if so, how. Research is being conducted to find out if BuChE has any role in the development of the nervous system or its functioning. It is also yet to be found out whether BuChE, AChE or other esterases have any role in carcinogenesis or cell growth and death.8

The muscarinic clinical effects of cholinergic excess secondary to organophosphorus insecticide poisoning can be easily recollected using the mnemonics DUMBELS and SLUDGE.

- D | Defaecation
- U | Urination
- M | Miosis
- B | Bronchorrhea, bradycardia, bronchospasm
- E | Emesis
- L | Lacrimation
- S | Salivation
- G | GI pain

**Fig. 1:** Suicide percentages in states and union territories in 2019

**Table 1:** Means of suicide adopted  

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Means adopted</th>
<th>Percentage and number</th>
<th>2018</th>
<th>2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ingestion of sedative tablets</td>
<td>0.7% (939)</td>
<td>0.5% (753)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>“Drowning”</td>
<td>4.9% (6579)</td>
<td>5.2% (7208)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Fire/self-immolation</td>
<td>4.4% (5950)</td>
<td>3.8% (5234)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Firearms</td>
<td>0.4% (524)</td>
<td>0.3% (428)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>By hanging</td>
<td>51.5% (69306)</td>
<td>53.6% (74629)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>By poison</td>
<td>26.7% (36862)</td>
<td>25.8% (35882)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Self-imposed injuries</td>
<td>0.6% (772)</td>
<td>0.6% (828)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Jumping from heights</td>
<td>1.9% (2557)</td>
<td>1.5% (2034)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Runover by vehicles/trains by jumping into their paths</td>
<td>2.9% (3848)</td>
<td>2.4% (3337)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Electrocution</td>
<td>0.4% (565)</td>
<td>0.5% (752)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Other measures</td>
<td>5.7% (7617)</td>
<td>5.8% (8038)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Total</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

Bronchorrhea, bradycardia, and bronchospasm are also called the “killer Bs.” The nicotinic effects of cholinesterase inhibitors include muscle fasciculations, cramps, weakness, mydriasis, tachycardia, and hypertension. There are four syndromes related to organophosphate poisoning—acute poisoning comprising of symptoms already described, which can occur in various combinations; chronic toxicity, intermediate syndrome, and organophosphate-induced delayed neuropathy. The intermediate syndrome usually occurs 1–5 days after exposure. It need not occur in every case. It is characterized by paralysis of respiratory muscles, neck flexor muscles, and proximal limb muscles. During this phase, there is usually no other symptom or sign of excess cholinergic activity. Ventilatory support may be needed.
Fig. 2: State/union territory wise suicide rate 2019

Note:
Suicide Rate means Number of Suicides per One Lakh population.
It usually resolves within 7 days. Agricultural laborers who are exposed to these pesticides almost daily are susceptible to chronic toxicity. It presents as a symmetrical sensorimotor axonopathy. It usually starts with leg cramps and progresses to weakness and then paralysis, similar to Guillain-Barre syndrome. Extrapyramidal symptoms, memory loss, mood swings, cognitive impairment, peripheral neuropathy, and autonomic dysfunction are features of organophosphate-induced delayed neuropathy.  

**Glycemic Status at Presentation in Organophosphorus Poisoning**

In two previous studies from India, it was found that the blood glucose levels at presentation in acute OP poisoning are a reliable and cost-effective marker to estimate severity and outcome.  

It is noteworthy that the blood glucose level at presentation is a reliable correlate of the severity of poisoning. The authors concluded that the risk of mortality increases independently as the blood glucose level at presentation increases in those patients without diabetes mellitus. Furthermore, this association varies depending on the type of OP that was ingested. Ke et al.  

In another study conducted in Taiwan, investigators studied whether diabetes mellitus presenting with OP poisoning was assessed, and they found that blood glucose was higher in patients in the death group. They also concluded that hyperglycemia was an independent risk factor for poor prognosis.  

On the contrary, in a study conducted in Taiwan, investigators studied whether diabetes mellitus would affect the mortality of patients presenting with acute large-dose exposure to organophosphates and found that that might not be the case. They also concluded that the risk of developing new-onset diabetes mellitus may also be just minimal in the short term (Table 2).

**Table 2:** Studies correlating glycemic status with the severity of poisoning

<table>
<thead>
<tr>
<th>Study</th>
<th>Type</th>
<th>No. of the study participants</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raghapriya et al. 2018</td>
<td>Prospective analytical study</td>
<td>100</td>
<td>11% of the patients were hyperglycemic, and this group had the highest mortality (63.63%) and the highest ventilator requirement (100%).</td>
</tr>
<tr>
<td>Panda et al. 2015</td>
<td>Prospective analytical study</td>
<td>102</td>
<td>Transient hyperglycemia at the time of presentation showed a significant positive correlation with serum MDA as well as the dose of atropine given for treatment ($p &lt; 0.05$).</td>
</tr>
<tr>
<td>Moon et al. 2014</td>
<td>Retrospective observational case series</td>
<td>184</td>
<td>The patient group with the highest plasma glucose ($\geq 300$ mg/dL) at presentation had the highest case fatality rate.</td>
</tr>
<tr>
<td>Ke et al. 2015</td>
<td>Retrospective analytical study</td>
<td>116</td>
<td>Blood glucose at presentation was higher in the group who died ($p &lt; 0.01$).</td>
</tr>
<tr>
<td>Colak et al. 2014</td>
<td>Retrospective analytical study</td>
<td>71 (intensive care unit (ICU))</td>
<td>Intermediate syndrome occurred in 11 patients, and serum cholinesterase was significantly lower in all of them ($p &lt; 0.01$). They also had a significantly higher levels of plasma glucose at presentation ($p = 0.037$).</td>
</tr>
<tr>
<td>Liu et al. 2014</td>
<td>Prospective analytical study</td>
<td>118</td>
<td>The difference in mortality ($p = 0.117$) between patients with and without diabetes mellitus presenting with OP poisoning was not significant.</td>
</tr>
</tbody>
</table>
**Acute Organophosphate Poisoning and Creatine Phosphokinase (CPK) Levels**

It has been concluded in a few studies\(^{16,17}\) that plasma CPK levels can be an efficient biochemical marker for acute OP poisoning because it is easily available and also cost-effective. Serial measurement of its levels can help predict prognosis as well.

Serum CPK was evaluated as a predictor of intermediate syndrome, and a weak positive correlation was observed between its levels and poisoning severity. Elevated CPK levels were found in all patients 48 hours after poisoning, but three of them had levels higher than 1500 IU/L. These three developed the intermediate syndrome. Hence early recognition of the intermediate syndrome may be possible by periodic estimation of CPK, and this may help to prevent life-threatening complications.

Aygun et al. in 2007,\(^{19}\) in their study did not find any significant difference between the patients with severe and mild poisoning in the initial levels of aspartate aminotransferase and creatine kinase. They also could not find any rise of these markers in patients with intermediate syndrome either. It was therefore concluded that the serum levels of muscle enzymes measured in the first 24 hours may not predict whether the patients develop intermediate syndrome subsequently (Table 3).

**Serum Amylase and Lipase**

Sumathi et al.,\(^{20}\) compared various biochemical markers, including serum lipase, amylase, and CPK, with serum cholinesterase for their prognostic significance in OP poisoning. Serum lipase, amylase, and CPK were found to be negatively correlated with serum cholinesterase, which means that higher levels of these markers were associated with lower levels of plasma cholinesterase (that is, more severe poisoning). Out of these, the negative correlation between serum amylase and serum cholinesterase was statistically significant. Therefore, as expected, serum amylase provided the highest accuracy for estimating poisoning severity, followed by serum CPK and serum lipase. They concluded that hyperamylasemia is commonly associated with organophosphate poisoning and that serum amylase can be used as a prognostic indicator for OP poisoning.

Singh et al.\(^{21}\) measured the incidence of hyperamylasemia in organophosphate poisoning and found that mild hyperamylasemia was common in organophosphate poisoning.

Lee et al.\(^{22}\) published a study in 1998 looking into the significance of hyperamylasemia in organophosphate poisoning and whether it could be equated to a diagnosis of acute pancreatitis. They found that elevated amylase levels are common in severe organophosphate poisoning. But as we already know, elevated amylase levels alone cannot be used to diagnose pancreatitis, and therefore serum amylase is not reliable enough to diagnose OP-induced pancreatitis because its sensitivity and specificity are low. Serum lipase can be used to diagnose pancreatitis in those patients presenting with hyperamylasemia.

Dungdung et al.\(^{23}\) also studied the correlation of serum lipase, amylase, and plasma cholinesterase in acute OP poisoning. They conducted an observational study in a hospital on 100 patients who had acute OP poisoning. All age groups and both genders were taken up. All three of the above-said markers were measured at admission. Based on the serum cholinesterase levels at admission, patients got categorized into three groups. Group I had 20–50% of the normal serum cholinesterase levels. Group II had 10–20%, and group III had <10%. In all of them, serum lipase and amylase had a negative correlation with serum cholinesterase levels. It was also statistically significant. Serum amylase was found to have the highest diagnostic accuracy among them. A total of 10 patients died, and of them, six had <10% of normal plasma cholinesterase activity. Eight of these 10 patients also had hyperamylasemia. They concluded that hyperamylasemia is associated with organophosphate poisoning and that serum lipase and amylase and lipase can be used as prognostic indicators alongside serum cholinesterase. Serum amylase is better than serum lipase in predicting poisoning severity.

In 2021, Zobeiri from Iran also concluded that hyperamylasemia was associated with more severe clinical outcomes and higher fatality.\(^{24}\) In 2022, Patil et al. also concluded the same\(^{25}\) (Table 4).

**Leucocyte Count and Organophosphate Poisoning**

Kumar et al. in 2018\(^{26}\) concluded that leucocyte count is useful as a prognostic marker in organophosphorus poisoning. Ke et al.\(^{13}\) and others concluded that the acute physiology and chronic health evaluation (APACHE) II score, which uses leukocyte count, can be used to assess prognosis. Eizadi-Mood\(^{27}\) and others analyzed the prognostic value of each element of the APACHE II score in assessing the outcome of OP poisoning. APACHE II scores for patients who survived without intubation were much lower than those who survived after intubation and those who died. White blood cell (WBC) count in APACHE II was found to be prognostically valuable (Table 5).

**Serum Pseudocholinesterase (BuChE)**

Biomarkers are highly useful in disease diagnosis. Serum cholinesterase is one such marker which is decreased in OP

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**Table 3:** Studies correlating serum CPK with the severity of poisoning

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of study</th>
<th>No. of study participants</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Madboly et al. 2013(^{16})</td>
<td>Prospective analytical study</td>
<td>60</td>
<td>About 15% had severe poisoning. There was a highly significant correlation between CPK levels and poisoning severity.</td>
</tr>
<tr>
<td>Bhat latex2011</td>
<td>Prospective analytical study</td>
<td>63</td>
<td>A total of 14 patients had severe poisoning (POP score 8–11), and serum CPK levels, as well as the total dose of atropine, showed a positive correlation with severity.</td>
</tr>
<tr>
<td>Kumar et al. 2015(^{18})</td>
<td>Prospective analytical study</td>
<td>75</td>
<td>CPK levels showed only a weak positive correlation with poisoning severity ($r = 0.352$). Elevated CPK levels at 48 hours were found in all patients after poisoning, but three of them had levels higher than 1500 IU/L. These three developed the intermediate syndrome. Hence early recognition of the intermediate syndrome may be possible by periodic estimation of CPK, and this may help to prevent life-threatening complications.</td>
</tr>
<tr>
<td>Dursun Aygun et al. 2007(^{19})</td>
<td>Prospective analytical study</td>
<td>47</td>
<td>Did not find any significant difference between the patients with severe and mild poisoning in the initial levels of aspartate aminotransferase and creatine kinase. They also could not find any rise in these markers in patients with intermediate syndrome.</td>
</tr>
</tbody>
</table>
Serum BuChE measurements are very useful for rapid initial screening of organophosphate exposure and hence can help in protecting humans from overexposure to these pesticides. A study conducted by Xu et al. in 2010 attempted to evaluate the diagnostic value of plasma BuChE by comparing it with AChE. When the AChE activity was low, the activity of BuChE also became low correspondingly. The levels of both enzymes changed in a similar manner and coincided with clinical symptoms. They found that when the level of BuChE was 20% of normal, OP poisoning was of moderate severity, and when it reached <10%, the poisoning was severe. Different kinds of OP pesticides were taken into consideration to arrive at this conclusion.

**Newer Markers**

Serum β-glucuronidase has also been studied as a marker for OP poisoning severity by Beltagy et al., with higher levels found in more severe poisoning.

**Recent Advances**

Reactivators (oximes) have been used since the 1950s to treat OP poisoning, which helps to revive the AChE enzyme inhibited by OP compounds. But, the effectiveness and toxicity potential of these reactivators are still points of debate. Another new option being explored is enzyme therapy. Organophosphorus hydrolases have recently spiked interest. They are a group of enzymes that help to detoxify OP compounds in the body.
that have shown promise in detoxifying OP compounds. They have shown antidotal effects against some OP compounds in vivo in animal models. Stoichiometric bio scavengers and catalytic bio scavengers are two groups of enzymes studied, with examples, including plasma paraoxonase-1 and OP acid anhydrase.33

An alternative strategy which has been studied is serum BuChE reactivation. Organophosphates stoichiometrically inhibit BuChE without any toxicity. Reactivation of BuChE may allow it to bind to the circulating OP molecules before they can reach the target AchE enzyme.34 Recently, zirconium metal-organic polyhedra have also been studied for treatment.35

Liver markers were also studied for correlation with OP poisoning severity in Sri Lanka, and a positive correlation was found between higher hepatic transaminase levels and the severity of poisoning.36

**Discussion**

Organophosphates are commonly used for suicide by poisoning. Early recognition of the diagnosis and its severity will help in achieving a better outcome. Currently, serum AchE and pseudocholinesterase are widely accepted as biochemical markers to estimate OP poisoning severity. But the possibility of using a wide array of alternate, cheap and easily available markers exists, as evidenced by the studies mentioned above and using a combination of these markers may be better in terms of early identification of severe poisoning. In peripheral centers without access to costly investigations, these cheap markers may help in guiding an early referral to higher centers for severely poisoned patients. A comprehensive study comparing all these different markers has not been done so far, thereby emphasizing the need for such a study.

**Conclusion**

Various new, cheaper, and easily available biochemical markers have been identified as having the potential to act as surrogates for assessing the severity of organophosphate poisoning, and there is a scope for future studies to understand the utility.

**References**