"ASSOCIATION BETWEEN SERUM CHOLINESTERASE LEVELS AND CLINICAL OUTCOME IN PATIENTS OF ORGANOPHOSPHORUS COMPOUND POISONING – ONE YEAR HOSPITAL BASED LONGITUDINAL STUDY"

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ENDORSEMENT

This is to certify that the dissertation entitled **"ASSOCIATION BETWEEN SERUM CHOLINESTERASE LEVELS AND CLINICAL OUTCOME IN PATIENTS OF ORGANOPHOSPHORUS COMPOUND POISONING – ONE YEAR HOSPITAL BASED LONGITUDINAL STUDY"** is a bonafide research work done by **CANDIDATE REG NO. BG0113015**.

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LIST OF ABBREVIATIONS USED

ACh	-	Acetylcholine
AChE	-	Acetyl cholinesterase
APACHE II	-	Acute Physiology and Chronic Health Evaluation II
CNS	-	Central nervous system
CPK	-	Creatine phosphokinase
DEF	-	Tribufos
DNA	-	Deoxyribonucleic acid
e.g.	-	For example
ECG	-	Electrocardiogram
GCS	-	Glasgow Coma Scale
GI	-	Gastrointestinal
HETP	-	Hexaethyl tetraphosphate
hrs	-	Hours
i.e.	-	That is
ICU	-	Intensive care unit
IM	-	Intramuscular
IMS	-	Intermediate syndrome
LD	-	Lethal dose
LDH	-	Lactate dehydrogenase
mEq/L	-	Milli equivalents per liter
mg/dL	-	Milligrams per deciliter
mg/kg	-	Milligrams per kilogram
ml	-	Milliliter
mm	-	Millimeter
mRNA	-	Messenger ribonucleic acid

n	-	Total number
NPIC	-	National Poison Information Center
NTE	-	Neuropathy target esterase
OP	-	Organophosphorus
p	-	Probability
P=O	-	Phosphorous oxygen bond
P2AM	-	Pralidoxime
PAM	-	Pralidoxime
POP	-	Peradenya Organophosphorus Poisoning
RBCs	-	Red blood cells
SChE	-	Serum cholinesterase
SGOT	-	Serum glutamic oxaloacetic transaminase
SGPT	-	Serum glutamic pyruvic transaminase
TEPP	-	Tetraethyl pyrophosphate
U/L	-	Units per liter
WHO	-	World Health Organization

ABSTRACT

Background and objectives

The activity of serum cholinesterase decreases in organophosphate compound poisoning. This study was aimed to evaluate serum cholinesterase levels as a prognostic marker for patients with organophosphorus poisoning.

Methodology

The present one year hospital based longitudinal study was done on a total of 85 patients admitted with organophosphorus compound poisoning in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2014 to December 2014. The estimation of pseudocholinesterase levels was done at the time of admission, on the fifth day and at the time of discharge.

Results

Maximum number of cases had age < 30 years (55.29%) and the mean age was 35.28 ± 15.34 years. There was male preponderance with a male to female ratio of 2.4:1. The most commonly consumed compound was malathion (24.71%). Majority of the patients (62.35%) presented after 3 to 6 hours of consumption. The most common symptom was vomiting (87.06%) and POP score revealed moderate intoxication in 58.82% of the patients. Serum cholinesterase levels were profoundly low (≤ 2500 U/L) in 62.35% of the patients at admission, 65.33% on fifth day and 62.71% at the time of discharge. Acute renal failure was the most common complication, noted in 23.08% of the patients.

Mortality was noted in 15.29% of the patients and intermediate syndrome was the commonest cause (30.77%).

Conclusion and interpretation

There is a positive association of serum cholinesterase with hospital stay (first day levels and serial estimation); requirement of ventilatory support (first day levels); and outcome (serial estimation).

Keywords

Organophosphorus compound poisoning; Peradeniya Organophosphorus Poisoning (POP) Scale; Serum cholinesterase;

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INTRODUCTION

Organophosphorus (OP) compounds are a diverse group of chemicals used in both domestic and industrial settings. Organophosphate compounds are frequently used as pesticides in agricultural activities. Examples of organophosphates include insecticides (malathion, parathion, diazinon, fenthion, dichlorvos, chlorpyrifos, ethion), nerve gases (soman, sarin, tabun, VX), ophthalmic agents (echothiophate, isoflurophate), and antihelmintics (trichlorfon). Herbicides (tribufos [DEF], merphos) are tricresyl-phosphate containing industrial chemicals.[1,2]

Pesticide poisoning is a major public health problem in the developing world.[3] Millions of people are exposed to the danger of hazardous occupational practices and unsafe storage of pesticides.[4] However, it is deliberate self-poisoning which causes the great majority of deaths and places immense strain on hospital services, particularly in Asia.[4,5]

According to a World Health Organization report, three million cases of pesticide poisoning occur annually worldwide and most of them are in Asia, of which at least half are due to organophosphorus (OP) poisoning.[6,7]

Poisoning is a common method of suicide, especially in the developing world.[7] OP compounds are amongst the most common poisons used for deliberate self-poisoning in India and other parts of the world.[8-11] India being predominantly an agricultural country, OP compounds are used abundantly for farming in India. Hence, access to these hazardous chemical substances is easy. However, the exact incidence of OP poisoning in India is uncertain due to lack of data and proper reporting. Based

on the literature from India, rate of suicidal poisoning with OP compounds ranges from 10.3 to 43.8%.[12] Among OP poisoned patients in India, hospital mortality rate is reported to be as high as 20-70%.[12,13]

OP compounds increase the accumulation of acetylcholine in the synaptic gap through inhibition of Acetyl cholinesterase and decrease in the degradation of acetylcholine, thus leading to excessively increased cholinergic activity and the appearance of cholinergic symptoms. Oximes like pralidoxime cause Acetyl cholinesterases to reactivate by binding to OPCs that have been bound to acetylcholinesterases.[14]

The primary mechanism of action of organophosphate pesticides is inhibition of carboxyl ester hydrolases, particularly Acetyl cholinesterase (AChE). AChE is an enzyme that degrades the neurotransmitter acetylcholine (ACh) into choline and acetic acid. ACh is found in the central and peripheral nervous system, neuromuscular junctions, and red blood cells (RBCs). Organophosphates inactivate AChE by phosphorylating the serine hydroxyl group located at the active site of AChE. The phosphorylation occurs by loss of an organophosphate leaving group and establishment of a covalent bond with AChE. Once AChE has been inactivated, ACh accumulates throughout the nervous system, resulting in over stimulation of muscarinic and nicotinic receptors. Clinical effects are manifested via activation of the autonomic and central nervous systems and at nicotinic receptors on skeletal muscle.[2,15] OPs lead to undesired effects on the central nervous system, cardiovascular system, respiratory system, urogenital system, neuromuscular junction, and metabolic and endocrine systems. Muscarinic symptoms like miosis, bradycardia, bronchospasm, urine and fecal incontinence; and nicotinic symptoms

like muscle weakness, muscle fasciculations, tachycardia, loss of consciousness and globe vesicale occur.[2,16]

The Peradenya Organophosphorus Poisoning (POP) scale assesses the severity of the poisoning based on the symptoms at presentation and is simple to use.[17]

There are various factors that predict the outcome in acute organophosphate poisoning. The recognised predictors of poor outcome include initial blood pressure, Glasgow Coma Scale (GCS) score, serum cholinesterase (SChE) level, APACHE II (Acute Physiology and Chronic Health Evaluation II) score, and electrocardiogram findings (prolongation of QT interval).[18,19] Red blood cell cholinesterase (true cholinesterase) level is a sensitive indicator, but its estimation is difficult and is usually not done. SChE activity is often checked in organophosphate poisoned patients. The activity of serum cholinesterase decreases in OP poisoning cases and the sensitivity is close to 100% for cases with significant OP poisoning.[2]

However, the significance of estimation of serum cholinesterase activity to assess the severity of illness and prognosis in patients with OP compound poisoning is not conclusive in previous studies.[20,21] There is a certain amount of ambiguity in the current data as far as the usefulness of serum cholinesterase in prognosis of organophosphorus poisoning is concerned. This prompted us to demonstrate the value of serum cholinesterase levels as a prognostic marker for patients with organophosphorus poisoning.

OBJECTIVES

The objective of this study was “to study the association between serum cholinesterase levels and clinical outcome in patients of organophosphorus compound poisoning”.

REVIEW OF LITERATURE

ORGANOPHOSPHATE COMPOUNDS

Organophosphorus compounds (OPs) are the chemical compounds containing carbon-phosphorus bond. They are a large group of compounds having the potential to irreversibly inhibit the cholinesterases namely acetylcholinesterase, pseudocholinesterase and neuropathy target esterase (NTE) in humans and animals. The OP compounds are not only used as insecticides and pesticides, but also as chemical warfare agents, petroleum additives, and industrial plasticizers. Serious human exposure leads to both muscarinic (cholinergic) hyperstimulation and nicotinic receptor stimulation.[22]

Historical note

Organophosphate compounds were known to exist since early 1800s, when Lassaigne synthesized OP compounds. However, the earliest recorded description of their synthesis was by De Clermont in 1854. Subsequently, Michaelis in Germany and Arbusov in Russia described synthesis of a large number of OP compounds in early 1900s. However, the toxic effects of OP compounds were not recognized until 1932, when Lange and Von Kruger described the toxic effects of OP vapors. In Germany, Schrader's deep involvement in the systematic research of OP compounds led to the development of more than 2000 OP compounds including sarin, parathion, and paroxon. Sadly however, given the prevailing scenario at the time, i.e. World War II, Schrader's work was mainly oriented toward the synthesis of chemical warfare agents. After the war, the pharmacological properties of these agents were

published leading to the realization of their potential use as insecticides and pesticides. This eventually led to the development of many more new OP compounds and their main use as insecticides and pesticides worldwide.[22,23]

Contemporaneous to the development of OPs as insecticides, another group of OP compounds were developed for industrial use. These compounds are triaryl esters of phosphoric acid (e.g. tricresyl phosphate) and are now used as plasticizing agents, lead scavengers in gasoline, and as additives to hydraulic lubricants. Tri-orthocresyl phosphate was implicated in the enormous epidemic of Ginger-Jake paralysis in people who consumed contaminated alcoholic extract of Jamaican ginger in the United States in 1930s.[22]

Currently a total of about 890 active ingredients are registered as pesticides in USA and marketed in some 20,700 pesticide products.[24]

Epidemiology

Poisoning with OP compounds is a worldwide phenomenon. An estimated three million cases of pesticide poisoning occur worldwide, each year.[25] According to the World Health Organization (WHO), one million serious unintentional poisonings occur every year and an additional two million people are hospitalized for suicide attempts with pesticides.[3,22]

In India, OP compounds are among the most commonly used agents for suicidal poisoning.[26] Systematic community based data on the epidemiology of poisoning are not available from India. Hospital-based data suggest that barbiturates and copper sulfate were the commonly used agents in the years, 1972-1977; however, later they were replaced by OP compounds and aluminum phosphide.[22,27]

In 1995, National Poison Information Center (NPIC) was established at the All India Institute of Medical Sciences, New Delhi. Data on the pattern of poisonings in North India accumulated at this center suggest that suicidal poisoning with household agents is the most common modality of poisoning.[28]

The common household agents included OPs, carbamates, pyrethrinoids, rodenticides, detergents, and corrosives. Agricultural pesticides accounted for 12.8% of all cases of poisoning. Likewise, OPs caused most self-poisoning deaths in South and Central India.[29]

In a study from Andhra Pradesh, two-thirds of the patients were young adults aged less than 30 years; more than half were males, and attempted suicide was the most common intent for poisoning. Majority of deaths were due to poisoning with monocrotophos and endosulfan (an organochlorine).[30]

Another study from Sri Lanka showed that young age, lower socioeconomic strata, unemployment, unstable emotional relationships, psychiatric disorders, and alcohol abuse were the risk factors associated with self-intentional or suicidal pesticide poisoning and suicidal intent accounted for almost 85% cases of pesticide poisoning.[31]

Classification

Organophosphorus compounds are classified as:

I) By chemical structure[32]

a. Alkyl phosphates: HETP (hexaethyl tetraphosphate), TEPP (tetraethyl pyrophosphate/ tetron), fosvex, malathion, dementon, trichlorfon etc.

b. Aryl phosphates: Parathion, paraoxon, methyl-parathion, chlorthion, diazinon (Tik20) etc.

II) By toxicity[33]

a. Extremely toxic (LD50: 1 to 50 mg/kg), or highly toxic (LD50: 51 to 500 mg/kg): Chlorpyrifos, diazinon, dichlorvos, dimethoate, ethion, fenthion, methylparathion, monocrotophos, oxydemeton etc.

b. Moderately toxic (LD50: 501 to 5000 mg/kg), or slightly toxic (LD50: more than 5000 mg/kg): Acephate, malathion, penthoate, temephos, triazophos, trichlorphon etc.

Mechanism of action

Organophosphate compounds avidly bind to cholinesterase molecules. In human beings, the two principal cholinesterases are RBC or true cholinesterase (acetylcholinesterase) and serum cholinesterase (pseudocholinesterase).[24]

Normally the cholinesterases rapidly hydrolyze the neurotransmitter acetylcholine into inactive fragments of choline and acetic acid after the completion of neurochemical transmission. The neurotransmitter acetylcholine is present in the terminal endings of all postganglionic parasympathetic nerves, at myoneural junctions, and at both parasympathetic and sympathetic ganglia. The major toxicity of organophosphate compounds is by the covalent binding of phosphate radicals to the active sites of the cholinesterases, transforming them into enzymatically inert proteins.[24]

Organophosphates thus act as irreversible cholinesterase inhibitors because the organophosphate-cholinesterase bond is not spontaneously reversible without pharmacological intervention. The inhibition of cholinesterase activity leads to the accumulation of acetylcholine at synapses, causing overstimulation and subsequent disruption of transmission in both the central and peripheral nervous systems. Exposure to organophosphate compounds will therefore interfere with synaptic transmission peripherally at muscarinic neuroeffector junctions and nicotinic receptors within sympathetic ganglia and at skeletal myoneural junctions. This is accomplished by an overstimulation of acetylcholine receptor sites that leads to a variety of physiologic and metabolic derangements. Disruption of transmission also will occur at the acetylcholine receptor sites within the central nervous system.[24]

Mode of intoxication[34-37]

Most OP compounds are extremely lipophilic. They are therefore readily absorbed by passive diffusion across lung and gastrointestinal system or skin. Deliberate ingestion with suicidal intentions is common in developing countries,

where they are readily available and cheap. Dermal and mucous membrane absorption is slower but clinical poisoning can occur after prolonged exposure. Inhalation by accident may occur during spraying in improper conditions. Intoxication by inhalation may occur during chemical warfare and following accidents during storage particularly when stocks catch fire.

Other potential modes of OP poisoning include ingestion of adulterated fruits or cooking oil, and wearing contaminated clothing.

Choudary et al.[38] reported a foodborne outbreak of OP compound poisoning. The kitchen in which food was prepared had been sprayed earlier with malathion.

In 2011, Chopra et al.[39] found that the commonest route of poisoning was oral in the suicidal cases. The reasons for the suicide in males may include lack of employment, poverty, urbanization and various other stress related factors. In females, it may be due to marital disharmony.

Distribution and Storage

Following absorption, OP compounds accumulate rapidly in fat, liver, kidneys and salivary glands. The phosphorothioates (P=S), for example diazonin, parathion, and bromophos, are more lipophilic than phosphates (P=O), for example dichlorvos, and are therefore stored extensively in fat which may account for the prolonged intoxication and clinical relapse after apparent recovery which has been observed in poisoning from these OP insecticides. OP compounds generally are lipophilic and therefore cross the blood-brain barrier in most cases.[40]

Organophosphorus compound metabolism

After absorption in skin, GI tract or inhalation, the insecticides and their metabolites get distributed quickly especially in the liver, kidneys, adipose tissue and tissues rich in lipids. The plasma half life after a single administration is from few minutes to several hours which depends on type of compound and rate and amount of administration. Metabolism is mainly due to oxidation, by cytocrome-p-450 system and hydrolysis of ester bonds mediated by various esterases or paroxonases. Elimination mainly occurs via urine and faeces. Urinary and faecal elimination is usually rapid, 80-90% of most compounds being eliminated within 48 hrs. A very small proportion of OP compounds and their active forms are excreted unchanged in urine. Some compounds remain longer in body like fenthion and fenithrothion.[34,37,41]

These compounds and their active metabolites cause toxicity by inhibiting the function of acetylcholinesterase, the enzyme responsible for hydrolysing and inactivating the neurotransmitter acetylcholine. They also inhibit number of enzymes belonging to the group of carboxyl-esterases. The biological effects of OP compounds are a result of accumulation of endogenous acetylcholine at sites of cholinergic transmission.[34,37,41] Phosphorylated cholinesterases may undergo a dealkylation reaction of the OP moiety leading to “aged” enzyme, i.e. conversion of the inhibited enzyme into a non-reactivable form.[42] This process of “Ageing” normally takes 48-72 hours and this period is known as the “critical interval” because during this time administration of antidote is still effective in reversing the process. Once ageing is completed the enzyme cannot be reactivated. Plasma ChE recovers quickly within 4 weeks. Red cell AChE takes longer and may not be

restored. Affected AChE recovers at the rate of ~ 1% per day. Restoration of AChE activity occurs by slow denovo synthesis of free enzyme and also to some extent as a result of spontaneous dephosphorylation of the inhibited enzyme. The inactivation (phosphorylation) and reactivation (Dephosphorylation) vary considerably with different OP compounds, which accounts for differences in toxicity. Ageing has an important bearing on toxicity and treatment outcome.[34,37,41]

Organophosphorus compounds inhibit number of other enzymes such as lipases, trypsin and chymotrypsin which are phosphorylated by these compounds. The rate of reaction is slower compared to AChE and clinical consequences are not known. They also affect central nervous system, cardiovascular system, reproductive system and endocrine system. The acceptable daily intake is 0.02 mg/kg for malathion, and 0.004 mg/kg for parathion. (The amount of chemical which can be consumed every day for an individual life span with a certainty based on valuable evidence that no harm will result.)[34,37,41]

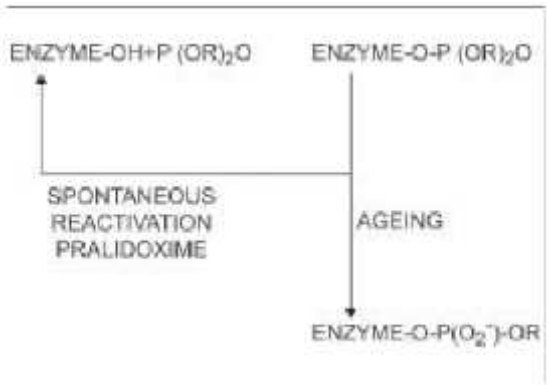


Figure 1. Biochemical basis of acute OP poisoning, its reaction with the enzyme acetylcholinesterase, the aging reaction, and reactivation following the administration of oximes[22]

Recovery from acute OP intoxication depends upon the reactivation of acetylcholinesterase OP complex. Although, the reactivation of the enzyme occurs spontaneously, this is a very slow process. Rapid reactivation can be achieved by pharmacological agents such as oximes. The latter compounds are nucleophilic agents and act as acetylcholinesterase reactivators.[22]

However, the action of oximes is limited by the aging reaction, a time-dependent process that hydrolyses the enzyme OP complex. As a result of aging, the enzyme is not susceptible to reactivation by oximes. As a general rule, the aging reaction occurs 48 to 72 hours after poisoning; hence at least theoretically, oximes are unlikely to provide therapeutic benefit beyond 48 to 72 hours after poisoning. In reality, different OP compounds have different aging half-lives. For instance, diethylphosphorylated acetylcholinesterase (a compound formed in poisoning by compounds such as parathion, chlorpyrifos, diazinon, and chlorfenvinphos) has a long aging half-life and can be effectively reactivated by the oximes over sustained periods of time. On the other hand, dimethylphosphorylated acetylcholinesterase (a compound formed in poisoning by compounds like malathion, paroxanmethyl, dimethoate, and oxydemeton) has a very short aging half-life and is not susceptible to the reactivating action of oximes over longer periods of time.[22,43]

Cholinergic transmission failure in OP poisoning involves both the muscarinic and nicotinic receptors and synapses. Acetylcholinesterase can be easily inhibited at the muscarinic synapses and nerve endings. However, failure of nicotinic transmission requires inhibition of at least 80% of the synaptic acetylcholinesterase. This is the basis for the nicotinic syndrome (intermediate syndrome) being seen later in the course of OP poisoning (usually, following the

acute muscarinic syndrome). Furthermore, the nicotinic syndrome occurs only in severe poisoning.[22,44]

Clinical manifestations

The clinical manifestation of OP poisoning depends on the agent, quantity and route of entry. Ingestion and inhalation result in more rapid development of symptoms than dermal exposure. After ingestion symptoms appear within 30-90 minutes and a maximum of 24 hrs in case of compounds which are highly lipophilic and which require metabolic bioactivation.[34,37]

Local Effects

GI symptoms appear first before the onset of systemic symptoms. Inhalation typically exhibits respiratory effects. Similarly, after ocular exposure symptoms generally begin in the eyes.[37]

Systemic Effects

Three well defined clinical phases are observed.[37]

- Initial cholinergic phase.
- The intermediate syndrome (IMS)
- Delayed polyneuropathy

The cholinergic phase[37,45,46]

It is mainly due to accumulation of Ach at the cholinergic synapses and may be classified into

1) Muscarinic (all postganglionic nerve endings)

2) Nicotinic (Autonomic ganglia and skeletal muscle end plates).

3) CNS manifestations (synapses in CNS)

Anxiety, restlessness, giddiness, emotional lability, slurred speech, ataxia, seizures, drowsiness, confusion, difficulty in concentration, headache, nightmares, insomnia, excessive dreaming, apathy, tremor, depression, generalized weakness, coma, absence of reflexes and Cheyne – Stokes respiration are the various possible CNS manifestations.

Muscarinic Features (Wadia Type 1 Syndrome)	**Nicotinic features (Wadia Type 2 Syndrome)**	**CNS features**
Miosis	Muscle fasciculations (straited)	Unconsciousness
Sweating / Diaphoresis	Paralysis	Confusion, fatigue
Bronchorrhea/ Bronchospasm	Muscle weakness	Toxic psychosis, seizures
Bradycardia	Hypertension	Respiratory depression
Hypotension	Tachycardia	Ataxia, dysarthria
Vomitting		Extra pyramidal features
Diarrhea		
Salivation		
Lacrimation		

The intermediate syndrome (IMS)

After apparent recovery from cholinergic crisis, muscle paralysis occurs (before the expected onset of delayed polyneuropathy).[47] This phenomenon has been identified as "Intermediate syndrome" (IMS). This is a type of paralysis first described by Wadia et al[36] in 1974 as the Wadia type II syndrome and later christened as "Intermediate syndrome" (IMS) by Senanayake, Karalliedde L.[40] The syndrome is of acute onset, seen within 24 to 96 hrs (1-4 days) after poisoning, affecting conscious patients without fasciculations or other cholinergic manifestations. The cardinal features of this syndrome is muscle weakness affecting predominantly proximal limb muscles and neck flexors.[37,45,48,49]

Delayed polyneuropathy

Though uncommon in India the neuropathy develops following a latent period of 2-4 weeks after the cholinergic crisis. The main clinical features are weakness of distal muscles of feet and hand. The weakness is preceded by pain and parasthesia of limbs. Wasting of distal muscles, particularly small muscles of the hand and those of anterior and peroneal compartments of the leg is an inevitable consequence. In some patients pyramidal tract signs appear after a few weeks or few months. Recovery is variable. The phosphorylation of an enzyme, "neuropathy target esterase" in nervous tissue is considered to be responsible for the polyneuropathy.[35,37,49]

Other effects of OP poisoning

Cardiovascular system

Bradycardia and low blood pressure occur as a result of muscarinic effect. Patients may also present with nicotinic features such as tachycardia and hypertension.[37]

Respiratory system

Respiratory arrest is a common terminal manifestation of OP poisoning. It can be recalled that muscarinic action produces increased bronchial secretions and bronchoconstriction. On the other hand nicotinic action produces intercostal and other respiratory muscle weakness leading to respiratory paralysis.[37]

Altered immunity to infection

Immunosuppression is associated with severe cholinergic stimulation either from a direct action of acetylcholine on the immune system or secondary to toxic chemical stress associated with cholinergic poisoning.[37]

Gastrointestinal system

After ingestion of organophosphate compound, the common initial symptoms may be increased salivation, nausea, vomiting, abdominal tightness and cramps. Other muscarinic manifestations include diarrhea, tenesmus and faecal incontinence.[50]

Effects on reproduction

Following organophosphorus poisoning in females, abortions are being reported. In late 20th century, several experimental and epidemiological studies regarding hormonal imbalance especially sex hormones leading to adverse developmental outcomes, including foetal death, intrauterine growth restriction, congenital malformations and male / female infertility have been published.[37]

Effects on temperature regulation

Several studies have noted derangement of temperature regulation in the form of hypothermia (incidence 7%). Some patients may experience fever lasting for many days, a biphasic response.[35,37]

Vocal card paralysis

In few patients vocal card paralysis was reported within 2 days.[35,37]

Effects on other systems

- Eyes: myopia and pigmentary degeneration of retina.
- Joints: arthritis
- Interference with mitochondrial oxidative metabolism.[35,37]

Changes in metabolism and endocrine activity

Transient hyperglycaemia and glycosuria are often found in severe OP poisoning. Absence of acetone bodies differentiates it from diabetic coma, except for coma in diabetic patients due to hyperosmolarity from excessive blood glucose.[37]

Diagnosis

Acute cholinergic crisis[37,51-53]

- History of ingestion of the compound
- Signs and symptoms
- Inhibition of cholinesterase activity
- Improvement after atropine and oxime therapy

Organophosphate poisoning is generally diagnosed clinically based on the characteristic symptoms and the history of exposure to OP agents. When diagnosis is not evident, a depressed serum or RBC cholinesterase level is helpful (<50%). If OP poisoning is suspected, therapy should never be withheld pending confirmation of lab values.[37,51-53]

Intermediate syndrome

The diagnosis is clinical and should be suspected when a patient who is recovering from the cholinergic crisis develops respiratory difficulty. The presence of muscle weakness in the absence of muscle fasciculations and other cholinergic features differentiates it from cholinergic crisis. The early onset of muscle weakness distinguishes the IMS from the delayed polyneuropathy, which appears 2-3 weeks after poisoning.[37,51-53]

Delayed polyneuropathy

History of intoxication with OP agents and the time of onset and distribution of muscle weakness differentiate from other causes of acute polyneuropathy.[37,51-53]

Management

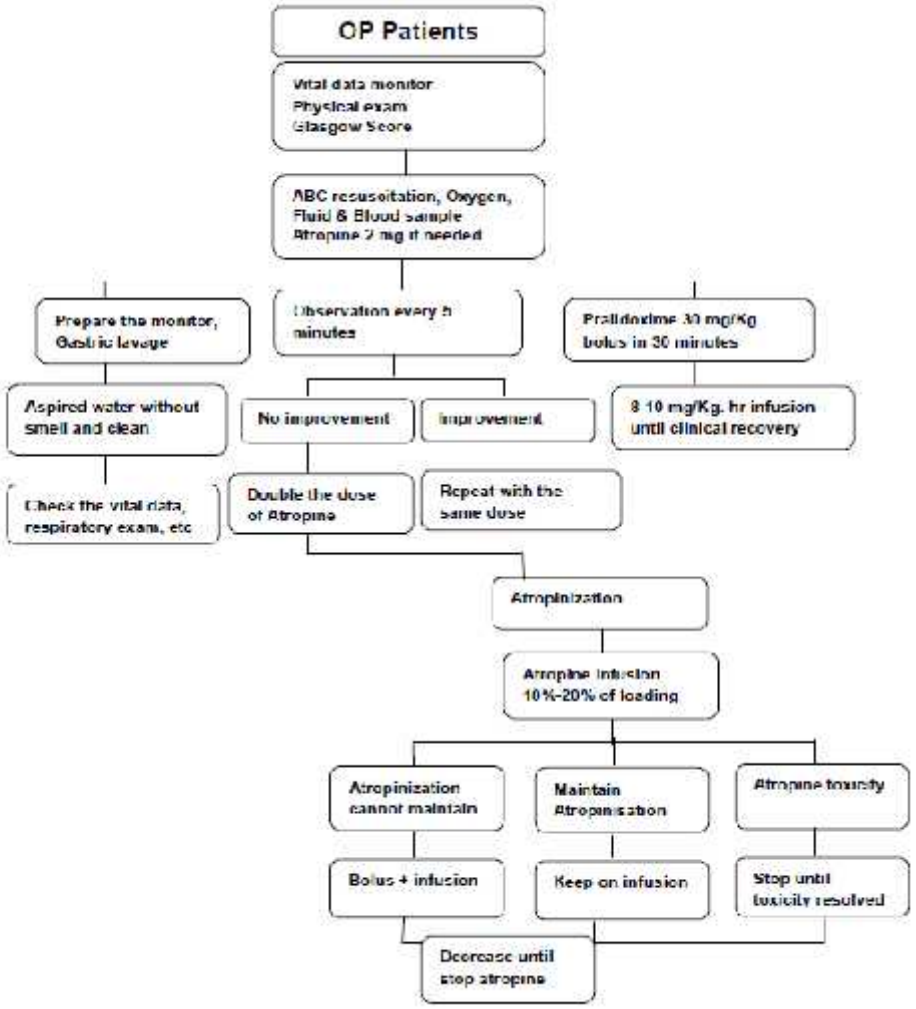


Figure 2. Treatment Protocol for OP Poisoned Patient[54]

<u>Decontamination</u>

Besides the decontamination at the poisoning site, in the emergency department all clothing, hair accessories are to be removed and placed in appropriate waste bags. The person is to be washed with copious amount of water and soap (OPs are hydrolyzed in an aqueous solution at high Ph).[54]

Skin folds, underside of fingernails and long hair require particular attention. Ocular decontamination is to be carried out by washing eyes with water/normal saline. Incontinent patient requires repeat wash.[54]

Attention should not be diverted from ABC of Cardiopulmonary resuscitation. Seizures are to be controlled by appropriate measures. Two IV lines should be secured and blood samples for haematological and biochemical analysis should be collected. ECG should be recorded. Laboured breathing, sweating, pin point pupil would suggest OP/carbamate poisoning. Atropine should be started immediately and should not be withheld even if oxygen is not available. There is no substantial evidence that giving atropine to a cyanosed patient would cause harm.[54]

In case clinical presentation is not clear Inj. Atropine 0.6 mg to 1.0 mg may be given IV. Increase in heart rate by more than 20-25 beats/min and flushing would suggest that the patient does not have significant cholinergic poisoning and further observation is required. While observing effects of atropine, give 500- 1000 ml of normal saline (10-20 ml/kg) over 10-20 minutes to compensate fluid loss due to sweating, diarrhoea and cholinergic hyper-secretion. There is no evidence that it will harm the patient with bronchorrhoea as long as atropine is being given.[54] History of alcohol ingestion should be enquired as well as possibility of other poison being consumed be ascertained.[54]

Active cooling and sedation

Cooling is indicated if the patient is febrile and climate is hot & humid. Agitated patients need to be sedated with Inj. Diazepam 10 mg IV slowly which can be repeated up to 30-40 mg/24 hrs. Diazepam will help in allaying anxiety, facilitate

gastric lavage, reduce damage to CNS,[54] diminish central respiratory failure[56] and control seizures.[54]

Gastric Decontamination

Forced emesis and syrup Ipecac have no role. Gastric lavage is indicated once patient is stabilized calm enough to give consent and in unconscious intubated patient. It is recommended to be repeated after 2-3 hrs.[54,57] Though it has been recommended that gastric lavage should be carried out within 1-2 hours of ingestion of OP/carbamate, it has been found to have beneficial effects even after 12 hrs of ingestion and being repeated thrice at an interval of 4 hrs.[58]

Repeat stomach wash will remove the residual poison if any and also some amount which is secreted into the stomach subsequently from fat stores. After aspirating the contents of stomach through stomach tube (if food particles are present)/Ryle's tube, water or normal saline in lots of 300 ml should be given and aspirated. Continue this procedure till the returning fluid is colourless and odourless. Ensure that no fluid is left inside the stomach by measuring the fluid taken out.[54]

Activated Charcoal

Though there is no evidence that either single dose or multiple dose regimens of active charcoal will result in benefit, yet a dose of charcoal (50 gm) can be left in the stomach.[59-61]

Transfer to ICU

Atropine and resuscitation measures are to be continued and vitals recorded while transferring to ICU. Patients with the following criteria may need ventilator support.[10]

- History of intake of large amount of poison
- Copious secretions
- Disturbed level of consciousness
- Signs of hypoventilation or respiratory obstruction by secretions

Hence intubation and ventilation facilities should be available in the ICU. A monitor should be connected to the patient and vital parameters including oxygen saturation are to be observed and recorded in OP observation sheet.[54]

Antidote

Anticholinergics are competitive antagonist to Ach and reverse all muscarinic activities both in the CNS and peripheral nervous system. Atropine is the only life saving antidote. Full and early atropinisation is an essential and simple part of early management. Clinical toxicology text books describe varied atropine regimens. The sum total of 38 regimes found in literature is, to give a bolus loading dose followed by boluses after a fixed time interval varying from 5-15-30 minutes till atropinisation or; Bolus loading dose followed by infusion. The latter regimen has shown better outcome.[54,62]

Although the benefit of infusion is not yet proven; this regime saves time, requires less observation, produces less fluctuation in plasma atropine concentration and makes weaning easier.[54]

The dose of atropine recommended is low (1-2 mg) so as to cater for milder cases and then rapidly escalated (doubled each time) to achieve atropinisation quite fast. Alternatively it is started with a bigger dose (5 mg) to ensure rapid atropinisation. Because of the danger of over atropinisation the former practice is preferred. The peak effect of atropine is seen within three minutes of an IV injection. Hence, one need not wait for more than five minutes before giving another bolus. Atropine administration by nebulisation also improves respiratory distress and oxygenation. It can also be given through endotracheal tube initially before securing a venous route.[54]

Criteria of Atropinisation

There are no comparative studies on markers for adequate atropinisation. Since patients usually die from respiratory or circulatory failure; air entry on chest auscultation, heart rate and blood pressure were given more importance than dilatation of pupil, rise in body temperature, dryness of mouth/skin.[54,55]

Target ends points for atropine therapy:

- Clear chest on auscultation with no wheeze
- Heart rate > 80 beats / min
- Pupil no longer pin point
- Dry axilla
- Systolic blood pressure > 80 mm of Hg.[54]

Atropine maintenance

Once the target end point is achieved it is to be maintained by atropine infusion. Infusion of atropine reduces the fluctuation in atropine concentration associated with repeated bolus doses. The rate of infusion is set at 10-20% of the total atropine required to load the patient every hour.[54]

Observation

Observations made initially after each atropine dose are entered in the observation sheet. Once atropinisation has been attained, those five parameters are to be monitored every 15 minutes initially which can be gradually increased to 1-2 hours depending on the state of atropinisation. Close observation and monitoring plays not only a vital role in the management but also can contribute to the learning process by gathering new symptoms and signs and can anticipate recurring cholinergic crisis which may occur with little notice.[54]

Atropine toxicity

Confusion, agitation, hyperthermia, ileus, tachycardia etc would suggest over atropinisation which would necessitate discontinuation of the atropine infusion, followed by frequent observation. When they settle down the infusion is to be started at 70- 80% of the previous rate. Hyperthermia is a serious complication in hot wards which needs prevention. To avoid preservative toxicity atropine should be reconstituted in normal saline and used.[54]

Duration of maintenance atropine therapy

This depends on the severity and response to therapy. Usually it is maintained for 24- 48 hrs or longer in severe cases, and gradually withdrawn over 3-5 days. Frequent observation is required to detect early signs of intermediate syndrome. Tidal volume and blood gases are to be measured.[54]

Guidelines for ventilator support[54]

I. Respiratory Gas Tensions
    i. Direct indices
        a. Arterial Oxygen Tension < 50 mm Hg on room air
        b. Arterial CO_2 Tension > 50 mm Hg in the absence of metabolic alkalosis
    ii. Derived Indices
        a. PaO_2 / FiO_2 < 250 mm of Hg
        b. PA –aO_2 (Pulmonary arterial – alveolar O_2 gradient) > 350 mm of Hg
        c. Vd / Vt > 0.6

II. Clinical – Respiratory rate (RR) > 35 breaths / min

III. Mechanical Indices
    i. Tidal Volumes <5 ml/kg
    ii. Vidal capacity <15 ml/kg
    iii. Maximum inspiratory force <- 25 cm of H_2O

Glycopyrrolate

Some studies have shown that glycopyrrolate is equally effective with fewer central nervous system side effects and better control of secretions. Since it does not enter CNS initial muscarinic signs like coma or drowsiness will not improve. Hence, it's use is recommended when there is copious secretion as an adjunct to atropine or when features of atropine toxicity like delirium etc are confused with CNS effects of poison or when atropine is not available. 7.5 mg of glycopyrrolate in 200ml of saline is started as infusion and is titrated to the desired effects of dry mucus membranes.[54] It has also been given at a dose of 0.2mg IM stat and repeated 6th hrly if required. Diphenhydramine can be used as an alternate centrally acting anticholinergic agent if atropine is not available.[63]

Magnesium Therapy

Magnesium therapy in addition to atropine and oximes has been found to have beneficial effects. The mechanism appears to be inhibition of AChE and OP antagonism.[64]

Cholinesterase Reactivators

These agents known as oximes get attached to the free anionic site of the enzyme ChE. The oxime end then reacts with the phosphorus atom of OP attached at the esteratic site of the enzyme. This oxime phosphate so formed diffuses away leaving the enzyme intact (Reactivated ChE). In addition to reactivation they also slow the ageing of the phosphorylated enzyme complex and have a direct action in converting the OP to a harmless compound.[65]

Reactivation of ChE is more marked in the skeletal muscle than at autonomic site and not at all in CNS (Do not enter CNS). Thus their use in OP poisoning is secondary to that of atropine. Oximes (Pralidoxime, Obidoxime) are widely used since 1955 in OP poisoning along with atropine within 24-48 hours post ingestion.[54]

WHO guidelines recommend giving a 30 mg/kg loading dose of Pralidoxime over 10-20 min followed by a continuous infusion of 8-10 mg/kg/hr until clinical recovery or seven days have elapsed whichever is later. Where obidoxime is available a loading dose of 250 mg is followed by an infusion of 750 mg every 24 hrs. Their efficacy however has been questioned over the last two decades by workers from Sri Lanka, South Africa, Taiwan, Iran and India. All these studies have been criticized either on the basis of non comparable groups of selected patients or inadequate doses of PAM.[57]

Though these studies have demonstrated clear reactivation of red cell acetylcholinesterase in diethyl OP pesticide poisoned patient (ageing in this compound AChE complex takes much longer than Dimethyl OPs) the reason for their failure to benefit was not apparent.[66]

Further studies on different regimens or different oximes have been recommended by these workers while proposing type of OP and it's coformulant, poison load, time to start of therapy and the dose of oxime being the limiting factors.[67]

Since the idea is sound and animal experiments suggest that oxime administration in OP poisoning is useful, it is recommended; preferably in moderate

to severe cases.[68] It is to be administered as early as possible post ingestion to offer benefit. Delayed presentation is not a contraindication.[54]

There are no definite dose recommendations. However, it has been established that the therapeutically effective oxime concentration in plasma is 4mg/litre though therapeutic effect has been demonstrated in lower concentration as well. In many studies pralidoxime has been used in a dosage of 2 gm loading followed by 1gm/h for 48h by infusion (high dose) with significant benefit, concluding that administration of pralidoxime in high dose and thereafter constant infusion is better than repeated bolus administration.[69]

In one study patients were randomized into two groups. Group I received a single bolus dose of 1gm P2AM at admission (Low dose group) followed by placebo infusion over the next four days. Group II received placebo bolus at admission followed by P2AM 12gm as a continuous infusion over the next four days. The number of patients who developed Intermediate syndrome was significantly lesser in the low dose group suggesting that the time of administration of P2AM is a crucial factor which determines response to therapy.[70]

Though the guidelines are not definite, it is clear that oximes are effective when given early and in high doses (dose to be adjusted depending on the salt used), that too a bolus dose offers benefit which is to be followed by infusion for a sufficient duration (therapeutic window for Diethyl AChe complex is 133h).[71]

Keeping these aspects in mind the guidelines followed by Southern hospitals network appears most appropriate and is recommended as Initial bolus dose of 2gm IV (30mg/kg) over 30 min(rapid administration leads to complications) which is to

be followed by 1 gm IV every 8 hours in mild to moderate poisoning and in severe poisoning cases 500 mg/hour(8-10mg/ kg/h)till clinical recovery (12-24h after atropine is no longer required or after the patient is extubated) or 7 days whichever is later. Administration of repeat bolus/ infusion is to be stopped based on clinical signs or plasma ChE levels.[54]

Though oximes are not recommended for Carbamate poisoning, their use should not be withheld in case of unknown cholinergic poisoning as definite harm to human beings has not been demonstrated.[55]

Ventilation

This is the most useful advancement made in the management of OP poisoning. Indications for ventilation have already been discussed. Regular and close observation during the initial course will guide us regarding when to ventilate. Succinylcholine is to be avoided (since it requires serum ChE for its metabolism). Non-depolarising neuromuscular blocking agents require higher doses to show effect.[54]

Furosemide

It is recommended if pulmonary oedema persists, even after full atropinisation.[54]

Antibiotics

Broad spectrum antibiotics are to be instituted as per antibiotics policy of the institution, considering the risk of infection due to frequent and multiple interventions.[54]

Agitation/Convulsion

Causes of agitation unrelated to pesticide toxicity like alcohol withdrawal, head injury etc are to be excluded. Role of Diazepam has already been described and is preferred over haloperidol.[54]

Injected OP poisoning

The effects OP poisoning are severe and requires higher doses of antidotes. Local tissue necrosis may need surgical intervention. [72]

SERUM CHOLINESTERASE LEVELS AND ORGANOPHOSPHORUS COMPOUND POISONING

Serum cholinesterase

In 1932 Stedman et al.[73,74] proposed the term cholinesterase to describe the enzyme which hydrolyses acetylcholine and other choline esters at a more rapid rate than non- choline esters. Other esterases occurring in animal tissues were found to be incapable of hydrolysing acetylcholine.

Eight years later, Alles & Hawes[73,75] showed that the cholinesterase found in human erythrocytes differed from that found in human plasma and in 1942 Mendel & Rudney showed that there were two types of cholinesterase.[73,76] The first of these is highly specific for acetylcholine and a few closely related esters; this they named true or specific cholinesterase. The other cholinesterase is capable of hydrolysing both choline and aliphatic esters and was thus named pseudo-, or nonspecific, cholinesterase.[74]

Later, Augustinsson introduced the term acetylcholinesterase for true cholinesterase, applying it to the enzyme found in erythrocytes as well as that found in connective tissue, i.e. nerve and muscle. In 1964, the Enzyme Commission gave acetylcholineasterase the systematic name acetylcholine acetylhydrolase (EC 3.1.1.7.) nonspecific (pseudo-) cholinesterase, acylcholine acylhydrolase (EC 3.1.1.8.).[74,77]

In 1979, the commission on Biochemical Nomenclature substantiated the names which had previously been suggested by Augustinsson. Nonspecific cholinesterase is synonymous with serum cholinesterase, plasma cholinesterase, pseudocholinesterase, butyrylcholinesterase or S-type cholinesterase. Thus cholinesterase should be regarded not as a single entity with identical properties whatever the enzyme's source but rather as a family of related enzymes with divergent properties dependent on the species, tissue and source.[74]

Physiology of the cholinesterases

Acetylcholinesterase has a vital function in the termination of synaptic transmission by hydrolysis of the neurotransmitter acetylcholine. On the other hand, the function of plasma cholinesterase is still the cause of some debate and, although there are several hypotheses, none has been universally accepted.[74]

In 1953, Lehman & Silk suggested that plasma cholinesterase prevented inhibition of acetyl- cholinesterase by removal of choline esters formed during metabolism thus indicating that plasma cholinesterase acts as a protector. Bergman & Wurzel postulated a role in the transmission of slow nerve impulses. Others have suggested a role for plasma cholinesterase in lipid metabolism or a regulatory role in choline homeostasis. It is known that both cholinesterases are closely associated with

membranes across which transport of water and ions take place and there is much evidence to support the cholinesterases as having a controlling role in the permeability of membranes.[74]

Zakut et al.[78] have demonstrated that efficient transcription occurs from the plasma cholinesterase gene in chorionic villi of 9 weeks gestation, but not from the acetylcholinesterase gene. They suggested that this finding supports the notion that chorionic villus growth and development, which itself could be dependent upon cholinergic signalling, may well need the protection of functioning plasma cholinesterase. Embryos which have defective plasma cholinesterase as a result of an inherited genotype could be at risk from exposure to unbuffered organophosphorus compounds, e.g. insecticides, and are unable to survive because of inhibited embryonic acetylcholinesterase. In this may lie the cause of some unexplained first trimester miscarriages, especially in Israel, a country in which there is a greater than average use of agricultural insecticides and an abundance of carriers of a defective cholinesterase gene.

The protective role of plasma cholinesterase *in utero* has implications for the more mature fetus as well. Simone *et al.*[79] have shown that plasma cholinesterase is present and functioning in the placenta at term, which has therefore the ability to metabolise cocaine. They postulate that this implies a metabolic barrier for the baby against such toxic materials, but concede that further studies need to be performed to determine whether placental variability in the biotransformation of cocaine is significant in fetal toxicology.

From the results of their experiments on isolated human lung preparations, Norel *et al.*[80] suggest a role for plasma cholinesterase in the co-regulation of the degradation of acetylcholine. They claim that neurotransmitter hydrolysis may be controlled via two enzymatic pathways: the primary system is acetylcholinesterase for the acetylcholine, which is held in the synaptic cleft and the secondary system, using plasma cholinesterase, for the extraneuronal acetylcholine which has diffused into the smooth muscle regions. This physiological finding agrees with the anatomical findings of Appleyard & Smith[81] who found that the acetylcholinesterase was present in Auerbach's plexus of a guinea-pig's ileum whereas the plasma cholinesterase was detected mainly in the smooth muscle.

Soreq *et al.*[82] reviewed the intriguing possibility that the cholinesterases play a part in tumourigenesis. There is abnormal expression of both the acetylcholinesterase and plasma cholinesterase genes at DNA, mRNA or the protein level in a variety of malignancies. Gene amplification, one of the common abnormal expressions, has even been found in premalignant conditions such as polycythaemia vera and is postulated to be an indicator in this condition for transformation to acute myeloid leukaemia.

Whilst speculating about the function of plasma cholinesterase, it should be remembered that the few individuals who apparently have no active enzyme are healthy, having no obvious metabolic disturbance. This should not argue against a role for plasma cholinesterase but rather the existence of alternative mechanisms in these subjects.[74]

Chemistry of the cholinesterases

Acetylcholinesterase is found in all excitable tissue, whether nerve or muscle, central or peripheral, cholinergic or adrenergic, motor or sensory, in most erythrocytes and in placental tissue. Plasma cholinesterase is found much more widely; in the central and peripheral nervous system, in most tissues especially the liver and, as its name implies, in the plasma. The two enzymes show different biochemical properties. Acetylcholinesterase shows high affinity for acetylcholine and a low affinity for noncholine esters. The specific substrate for acetylcholinesterase is acetyl-methylcholine. Plasma cholinesterase has a lower affinity for acetylcholine but unlike AChE is not inhibited by higher concentrations of acetylcholine. The hydrolysis rate of aliphatic choline esters increases with increasing length of the acyl chain up to *n*-butyryl. The specific substrate for this enzyme is benzoylcholine.[74]

The activity of plasma cholinesterase can be measured by adding plasma to benzoylcholine and following the reaction spectrophotometrically. This is the most straight- forward method and is that employed by the Cholinesterase Research Unit. The normal range of activity of plasma cholinesterase used at this unit is 0.8-1.2, the units being micromols of benzoylcholine hydrolysed per min per ml of plasma. A typical value for the activity of cholinesterase in an individual who is homozygous for the atypical gene would be about 0.4 units but this by no means defines the phenotype; there could be many causes of such a result. To clarify the phenotype, the reaction is carried out in the presence of inhibitors to this reaction such as dibucaine (cinchocaine, nupercaine), which is an amide local anaesthetic, sodium fluoride and a specific inhibitor known as Ro2-0683.[74]

Newer techniques include the use of enzyme-linked immunosorbent assays and DNA amplification and sequencing utilising the polymerase chain reaction.[74]

Profile of plasma cholinesterase

Plasma cholinesterase is synthesised in the liver and there is a relationship between the decreases in plasma cholinesterase and serum albumin in chronic liver disease. However, these two proteins are not interdependent and both have been used separately as markers of liver function. Although it is assumed that if there is a low serum albumin there will be a low plasma cholinesterase, e.g. during pregnancy, this does not always follow. There are reports of normal levels of albumin being found in individuals lacking in plasma cholinesterase and in the nephrotic syndrome hypo-albuminaemia and a high plasma cholinesterase concentration regularly coexist.[74]

Plasma cholinesterase is a complex molecule comprising four identical subunits, each subunit consisting of a polypeptide chain of 574 aminoacids and nine carbohydrate chains. The molecular weight of each subunit is about 85,000.[74]

The structure of the tetramer, including the complete aminoacid sequence and the whereabouts of the disulphide bonds, was determined by Lockridge *et al.* in 1987.[83] There are two active sites in plasma cholinesterase known as the anionic site and the esteratic (catalytic) site. It is this esteratic site which actually combines with the carbonyl group of the ester linkage and is responsible for the hydrolysis of the ester bond.[74]

The enzyme is thought to be stable both *in vivo* and *in vitro*. Evidence that there is a persistence of activity in stored frozen plasma is given by a case report of an infant, homozygous for the atypical gene, who was apnoeic for 7.5 h following

administration of suxamethonium. He was treated with 40 ml of fresh frozen plasma over 30 min with an almost immediate return of neuromuscular function.[74]

There is minimal change in enzyme activity in whole blood stored at 4°C for 30 days.[74]

Lovely *et al.*[84] describe a homozygote for the atypical gene, who had previously shown no sensitivity to suxamethonium, showing a typical persistence of neuromuscular blockade following a second exposure. This patient had been transfused with two units of packed red blood cells during the initial operation. They claim that the patient's enzymatic defect may have been masked by the plasma cholinesterase which was present in the transfused blood. This stability *in vitro* means that specimens sent by post to the Cholinesterase Research Unit will not have lost enzymatic activity by the time they arrive for analysis. *In vivo*, there is little variation in plasma cholinesterase activity in healthy adults when measured at regular intervals over a period of 5 years.[74]

The half-life of plasma cholinesterase can be calculated from the decrease in enzymatic activity after treatment of an anenzymatic patient with either plasma or purified cholinesterase. Another possibility is to measure the increase in enzyme activity following the inhibition of the cholinesterase after exposure to organophosphorus com- pounds or removal of plasma cholinesterase following plasmapheresis. The half-lives thus calculated varied from 45 h to 16 days.[74]

Causes of variation in plasma cholinesterase

The catalytic activity of plasma cholinesterase can be abnormal, being either increased or decreased. The cause of these variations can be physiological, acquired or inherited.[74]

Normal plasma cholinesterase activity shows a wide range in healthy adults. In a study by Callaway *et al.*[85] it was shown to be between 57% and 143% of the mean. No sex or age-related differences were found. (It should be noted that all the subjects used in this study were only known to be healthy but the samples almost certainly included some genotypically aberrant individuals.) The wide variation in enzyme activity shows a flat Gaussian distribution. More recent surveys show that males have a higher activity than females, but this may be explained by gender-related differences in muscle mass and extracellular fluid volume. There is no influence of age upon plasma cholinesterase activity in the adult population but this is not so for infants and children.[86]

Normal ranges of RBC and serum cholinesterase vary widely between individuals (and even in the same individual at different times). Because of this, a person who usually has a "high-normal" level of cholinesterase could be significantly toxic but his or her cholinesterase level could decrease only into the "low-normal" range.. Thus, the toxic patient would have a falsely normal test result.[86] (One author indicates that for serum cholinesterase there is a 300% difference between the lower and upper normal values.)[87] Thus, unless pre-exposure levels are available for comparison, only a level of inhibition greater than that due to

interindividual variability (about 25% for RBC cholinesterase) can be considered significant.[86]

Errors in laboratory results can also occur if samples are stored at room temperature, because cholinesterase bound to un-aged inhibitors can undergo significant spontaneous reactivation. Other Causes of Cholinesterase Level Abnormalities are as shown in the below table.[86]

	RBS Cholinesterase	Serum Cholinesterase
Low Levels	• Antimalarial drugs • Oral contraceptives • Some anemias	• Acute infections • Benzalkonium salts • Carbon disulfide • Chronic debilitating diseases • Ciguatoxins • Cocaine • Codeine • Dermatomyositis • Genetic deficiency (3% of individuals) • Hepatic parenchymal disease • Malnutrition • Morphine • Pregnancy • Oral contraceptives • Organic mercucy compounds • Solanines • Some anemias • Succinylcholine • Use of gray-top blood collection tubes or those containing fluoride
High levels		• Nephrotic Syndrome

Serum cholinesterase levels in organophosphorus compound poisoning

Organophosphorus compounds and carbamate pesticides are designed to inhibit acetylcholinesterase (AChE) and this enzyme has been used the most in enzymatic detection of these pesticides. Many enzymes used for the detection of pesticides are inhibited by the pesticide and the extent of inhibition is correlated to the concentration of the analyte. Other enzymatic methods such as the organophosphorus hydrolase assay use the analyte as a substrate, with the result that a positive signal is generated through the production of hydrolysis products rather than merely the inhibition of the enzyme.[88]

A study by Evtugyn *et al*.[89] gives a good overview on different enzymes that can be used for detection of toxicants. AChE catalyzes the hydrolysis of acetylcholine, which is a neurotransmitter in the synaptic membrane to prevent its accumulation. 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 the serine residue of AChE. This irreversible inactivation leads to an excess accumulation of acetylcholine in the peripheral and central nervous system causing cholinergic manifestations. At high doses, there is depression of the respiratory centre in the brain, followed by peripheral neuromuscular blockade causing respiratory paralysis and death. AChE gene polymorphisms have been studied extensively in the recent past mainly due to the interest in treatment of Alzheimer's disease. However, AChE is a highly conserved molecule, and only a few naturally occurring genetic polymorphisms have been reported in the human gene.[88]

SChE levels have been used for estimating the degree of inhibition of the enzyme to assess the severity of poisoning according to Proudfoot criteria. Serial estimation of SChE levels can detect the persistent enzyme inhibition. In most of the poisoning cases, the type of OP poison and the quantity consumed by the patient are unknown necessitating the need of serial estimation of serum AChE. There are a few studies in which serial SChE estimation was done in first few days of poisoning where the likelihood of developing life-threatening complications is considerably high.[91]

OP compounds exert their toxic manifestations by irreversibly inhibiting the AChE, SChE and neuropathy target esterase leading to accumulation of acetylcholine at the synapse. Thus, hyperstimulation of the central and peripheral nervous systems results in cholinergic crisis. The resulting muscarinic and nicotinic symptoms may continue for days or months until the cholinesterase enzyme gets reactivated. Estimation of OP exposure by cholinesterase levels helps to establish early diagnosis and thereby institute immediate treatment plan.[91] However, Eddleston et al.[92] believed that cholinesterase level estimation at a single time-point lacks sensitivity and specificity and thus might not be related to the severity of poisoning.

SChE activity has a high degree of variability from person to person. SChE is subject to high degree of variation induced by hereditary deficiency of this enzyme, liver function, malnutrition, iron deficiency anaemia, drugs such as cocaine, morphine, codeine and succinylcholine making this enzyme a less-than-perfect biomarker for organophosphate poisoning if baseline levels are unknown in an individual. A single measurement of SChE activity may have poor prognostic value

but serial SChE estimation is better in predicting outcome. According to a study, the absence of elevating SChE activity level within 48 hours of poisoning appears to be associated with a higher mortality in acute organophosphate poisoning patients.[93]

Literature

In a study conducted at Bir Hospital, National Academy of medical sciences, Kathmandu between August 2004 to September 2005, 50 patients were grouped into mild, moderate and severe poisoning groups according to the POP scale (Peradenya organophosphorus poisoning scale). The severity of poisoning directly correlated with serum cholinesterase level ($p<0.001$). There were 26% patients in moderate poisoning and only 4% patients in severe poisoning, but a total of 14% of the patients died indicating that further studies to evaluate the factors likely to cause deaths (e.g. co-morbidities) are required to clarify the correlation with mortality.[94]

In another study conducted at three hospitals in Sri Lanka between March 2002 and December 2003, 91 patients with proven dimethoate and 208 patients with proven chlorpyrifos self-poisoning were included. (This method was adopted because previous studies had been confounded by the inclusion of multiple insecticides with differing inhibitory kinetics.) It was found that plasma butyrlcholinesterase (pseudocholinesterase) levels can provide useful information but it must be interpreted carefully as its sensitivity and specificity for various insecticides is different.[95]

A study conducted at JSS Medical College, Mysore included 37 patients with history of organophosphorus poisoning, without any underlying diseases between January 2009 and December 2010. A retrospective analysis of the data with respect

to clinical manifestation at presentation, serum acetylcholinesterase activity results, details of patient management and outcome was done. It was found that serial measurement of serum acetylcholinesterase levels can be useful in predicting length of ICU stay, duration of mechanical ventilation and prognosis of the patient with organophosphorus poisoning.[88]

In another study conducted at Guru Govind Hospital Jamnagar, Gujrat between October 2010 and March 2011, fifty consecutive patients of acute organophosphorus poisoning were included. It was found that serum cholinesterase level is associated with organophosphorus poisoning ($p<0.05$). It was also found that patients with higher clinical admission POP (Peradenya organophosphorus poisoning) score had high requirement of ventilator support as compared to patients with low score ($p<0.05$).[96]

In a study conducted at University Hospital of Monastir, Tunisia from November 1989 to November 1992, thirty patients with the diagnosis of acute organophosphorus poisoning were included. Comparison of serum cholinesterase level in mechanically ventilated and non-mechanically ventilated patients did not reveal any significant difference. No correlation was found between serum cholinesterase level and the total atropine requirement. Hence it was inferred that serum cholinesterase levels have no prognostic value in acute organophosphorus poisoning.[97]

METHODOLOGY

This study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2014 to December 2014.

Study design and duration

A one year hospital based longitudinal study.

Study period

The present study was conducted from January 2014 to December 2014.

Place

The present study was conducted in Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum a tertiary care teaching hospital attached to Jawaharlal Nehru Medical College, Belgaum.

Source of Data

The study comprised of patients with organophosphorus compound poisoning admitted in the wards and ICUs of the Department of General Medicine.

Sample size

A total of 85 patients with organophosphorus compound poisoning were studied.

Sampling procedure

The total number of organophosphorus poisoning cases in the last three years was 220 according to the Department of Medical Records, KLES Dr. Prabhakar Kore Hospital and Research Centre, Belgaum. Considering 80% of the annual average number of cases with organophosphorus compound poisoning during the last three years, the sample size was determined as a minimum of 60 cases. However during the study period 85 cases were admitted with organophosphorus compound poisoning all of whom were included in the study.

Selection criteria

Inclusion

- Patients with suspected organophosphorus compound poisoning.

Exclusion

- Patients with other conditions which interfere with pseudocholinesterase estimation;
    - Chronic liver disease
    - Burns
    - Nephrotic syndrome
    - Pregnancy

Ethical clearance

Prior to the commencement, the ethical clearance was obtained from Institutional Ethics Committee, Jawaharlal Nehru Medical College, Belgaum.

Informed Consent

All the patients fulfilling selection criteria were explained about the nature of study and a written informed consent was obtained before enrollment (Annexure I). In case of patients with altered mental status, the relatives of the patients were briefed about the nature of study and written informed consent was obtained.

Method of collection of data

Demographic data such as age and sex were recorded. Patients / relatives were interviewed for chief complaints and past history. History of organophosphorus compound including type of organophosphorus compound, quantity consumed, route of exposure, intention of consumption, were noted as reported by either the patient or informant. A thorough physical, clinical and systemic examination was carried out. Based on these findings Peradeniya Organophosphorus Poisoning (POP) Scale clinical criteria score was calculated. These findings were recorded on a predesigned and pretested proforma (Annexure II).

Investigations

The selected patients underwent the following investigations.

- Complete blood count
- Urine routine
- Liver function tests
- Renal function tests
- Serum electrolyte levels
- Serum cholinesterase levels

- Electrocardiogram

Study variables

The patients were evaluated for following study variables

Severity of organophosphate compound poisoning

The severity of organophosphate compound poisoning was determined by Peradeniya Organophosphorus Poisoning (POP) Scale clinical criteria score. The POP scale assesses the severity of the poisoning based on the symptoms at presentation and is simple to use.[17]

Peradeniya Organophosphorus Poisoning (POP) Scale Clinical criteria Score

Variables	Findings	Score
Respiratory Rate	Normal (<20 /minute)	0
	Tachypnoea (>20 / minute)	1
	Tachypnoea (>20 / minute with central cyanosis)	2
Heart rate	Normal (>60 /minute)	0
	Bradycardia (41-60 /minute)	1
	Bradycardia (<40 /minute)	2
Level of consciousness	Conscious and rationale	0
	Impaired response to verbal commands	1
	No response to verbal command	2
Fasciculation	None	0
	Present - Generalised or continuous	1
	Both – Generalised and continuous	2
Pupil size	> 2 mm	0
	< 2 mm	1
	Pin point	2
Seizures	Absent	0
	Present	1

Interpretation of POP score

- Mild poisoning - A score of 0 to 3
- Moderate poisoning - A score of 4 to 7
- Severe poisoning - A score of 8 to 11

Serum cholinesterase levels

The estimation of serum cholinesterase levels was done by PCHE method using Flex reagent cartridge manufactured by Siemens Dimension clinical chemistry system. Pseudocholinesterase levels were estimated at the time of admission and during follow up of the patient on the fifth day of hospital stay as well as at the time of discharge. The serum cholinesterase levels between 7000 to 19000 U/L were regarded as normal.[98] The trend of change in serum cholinesterase levels from admission to discharge was also noted.

Ventilatory support and duration of ventilation

Number of patients requiring ventilation and duration of ventilatory support in each patient were noted.

Complications

Patients were monitored for the complications during their stay in the hospital.

Length of stay in the hospital

The duration of hospital stay was recorded.

Outcome

Patients were evaluated for the outcome as survivors and non survivors.

Statistical analysis

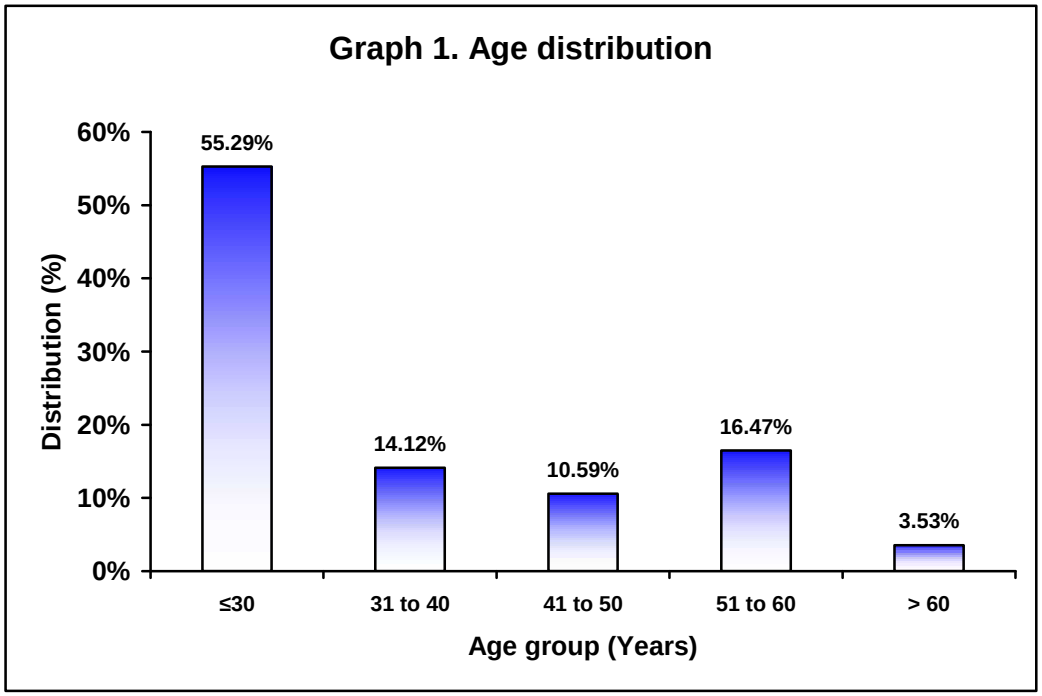
The data obtained was coded and entered into Microsoft Excel Worksheet (Annexure III). Data was analysed using SPSS statistical software version 20.0 The categorical data was expressed as rates, ratios and proportions and comparison was done using either chi-square test or Fisher's exact test. The continuous data was expressed as mean ± standard deviation (SD) and comparison was done using independent sample 't' test. A probability value ('p' value) of less than or equal to 0.05 was considered as statistically significant.

RESULTS

The present one year hospital based longitudinal study titled “Association between serum cholinesterase levels and clinical outcome in patients of organophosphorus compound poisoning” was carried out in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. During the study period from January 2014 to December 2014, a total of 85 patients admitted with organophosphorus compound poisoning were studied. The findings / observations and final results are tabulated as below.

Table 1. Age distribution

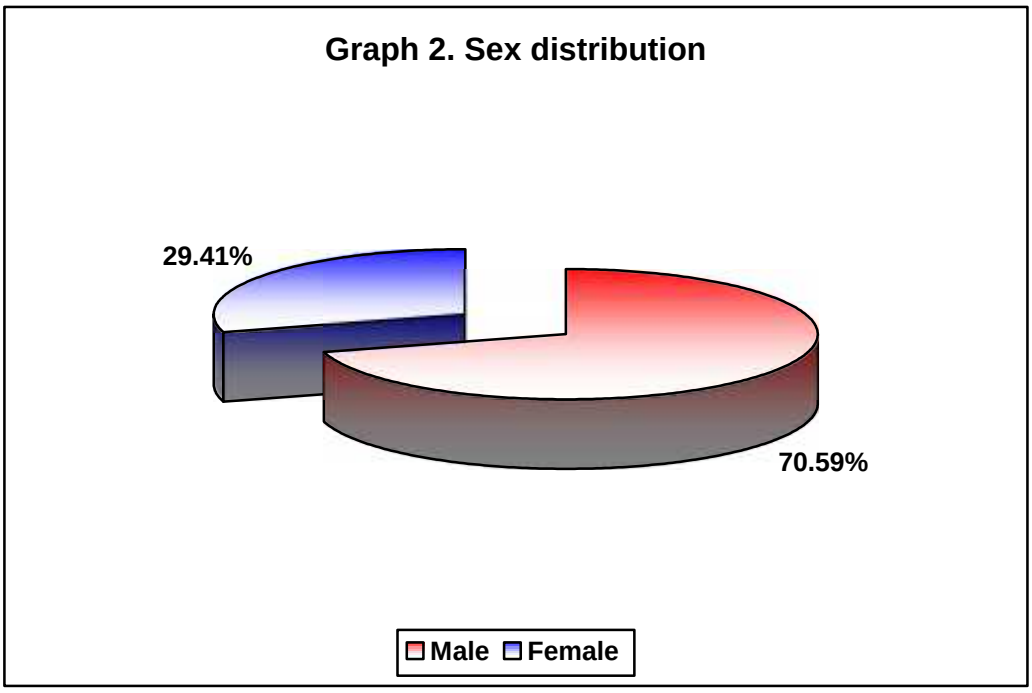
Age group (Years)	Distribution (n=85)	
	Number	**Percentage**
≤30	47	55.29
31 to 40	12	14.12
41 to 50	9	10.59
51 to 60	14	16.47
> 60	3	3.53
Total	**85**	**100.00**



Patients age ranged from 18 to 87 years, maximum number of cases were in the age group of below 30 years that is 47 patients (55.29%), between 51-60 years 14 cases (16.47%), 31-40 years 12 cases (14.12%), 41-50years 9 cases (10.59%) and only 3 cases (3.53%) in the age group of more than 60 years.

Table 2. Sex distribution

Sex	Distribution (n=85)	
	Number	**Percentage**
Male	60	70.59
Female	25	29.41
Total	**85**	**100.00**

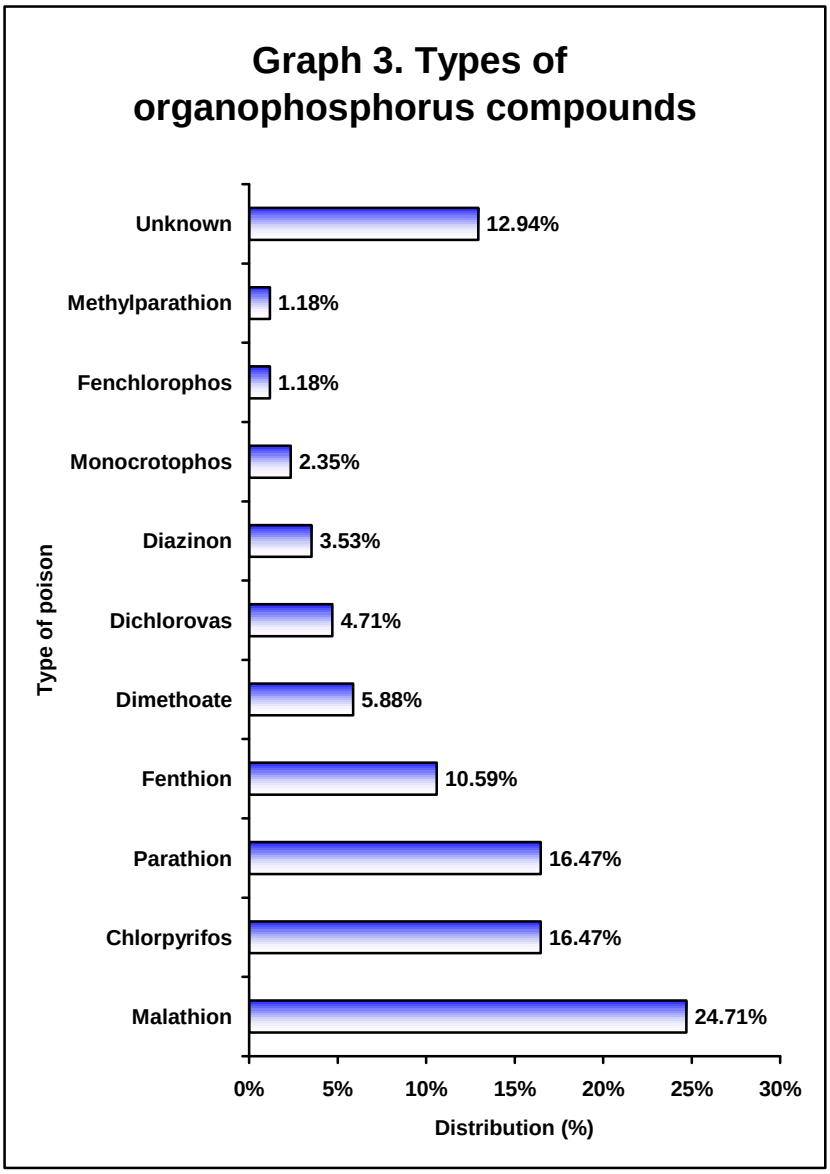


Out of 85 patients 60 (70.59%) were males and 25 patients (29.41%) were females, accounting a ratio of male to female 2.4:1.

Inference: Male preponderance was observed.

Table 3. Types of organophosphorus compounds

Type of poison	Distribution (n=85)	
	Number	**Percentage**
Malathion	21	24.71
Chlorpyrifos	14	16.47
Parathion	14	16.47
Fenthion	9	10.59
Dimethoate	5	5.88
Dichlorovas	4	4.71
Diazinon	3	3.53
Monocrotophos	2	2.35
Fenchlorophos	1	1.18
Methylparathion	1	1.18
Unknown	11	12.94
Total	**85**	**100.00**

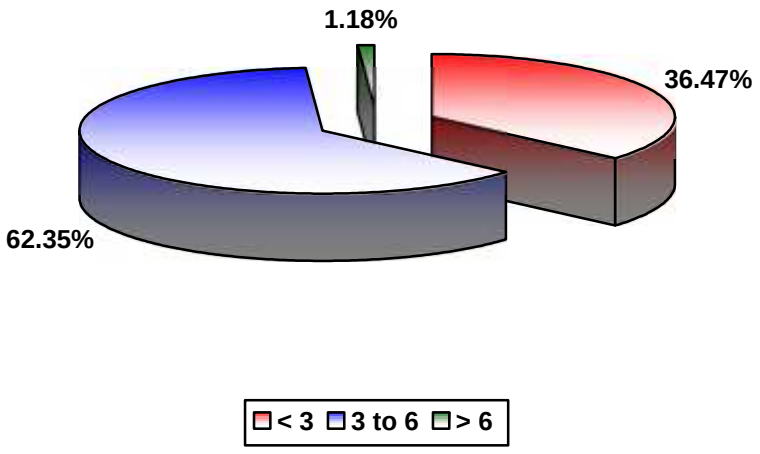


In the present study we observed that 21 patients (24.71%) had consumed malathion, 14 patients (16.47%) had consumed chlorpyrifos, 14 patients (16.47%) parathion, 9 patients (10.59%) fenthion, 5 patients (5.88%) dimethoate, 4 patients (4.71%) dichlorovas, 3 patients (3.53%) diazinon, 2 patients (2.35%) monocrotophos, 1 patient (1.18%) fenchlorophos, 1 patient (1.18%) methylparathion and in 11 patients (12.94%) the compound was unknown.

Table 4. Time interval from consumption of organophosphorus compound to arrival at hospital

Time (hours)	Distribution (n=85)	
	Number	Percentage
< 3	31	36.47
3 to 6	53	62.35
> 6	1	1.18
Total	**85**	**100.00**

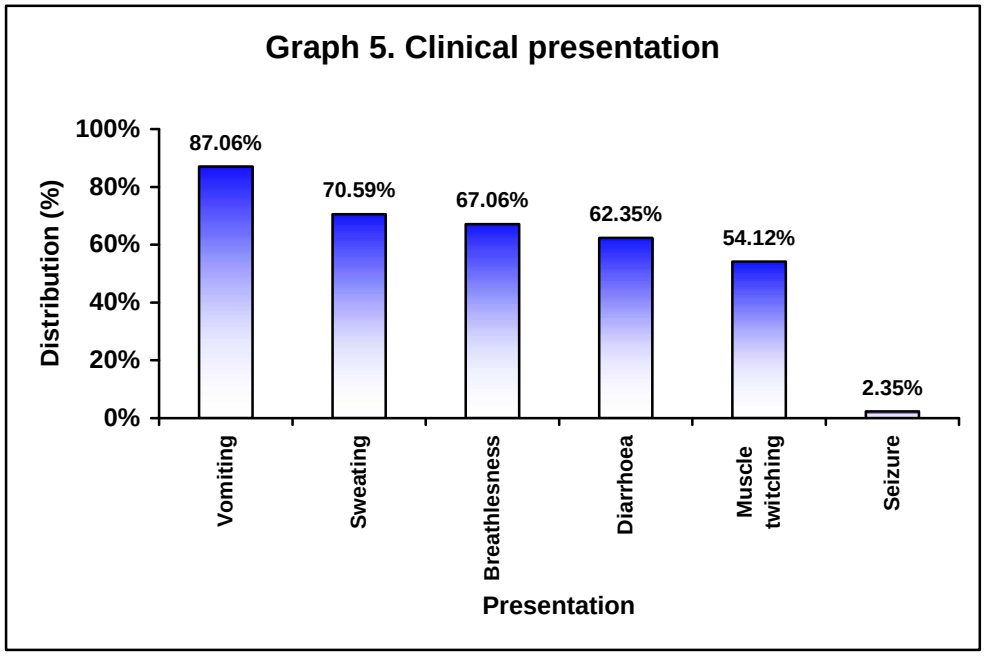
Graph 4. Time interval from consumption of organophosphorus compound to arrival at hospital



We observed that most of the patients 53 (62.35%) arrived between 3 to 6 hours of consumption, 31 patients (36.47%) arrived within less than 3 hours and only one patient arrived after 6 hours.

Table 5. Clinical presentation

Presentation (symptoms)	Distribution (n=85)	
	Number	Percentage
Vomiting	74	87.06
Sweating	60	70.59
Breathlessness	57	67.06
Diarrhoea	53	62.35
Muscle twitching	46	54.12
Seizure	2	2.35



In our study majority of patients presented with one or the other symptoms of poisoning. 74 patients (87.06%) had vomiting, sweating 60 patients (70.59%), breathlessness 57 patients (67.06%), diarrhoea 53 patients (62.35%), muscle twitching (fasciculations) 46 patients (54.12%) and seizures 2 patients (2.35%)

Table 6. Clinical presentation based on Peradeniya organophosphorus poisoning (POP) score

Variables	Findings	Distribution (n=85)		Score
		Number	Percentage	
Respiratory	Tachypnoea	58	68.24	1-2
Rate	Normal	27	31.76	0
	Total	**85**	**100.00**	
Heart rate	Bradycardia	60	70.59	1-2
	Tachycardia	6	7.06	0
	Normal	19	22.35	0
	Total	**85**	**100.00**	
Level of	Conscious and rationale	11	12.94	0
consciousness	Impaired response to verbal commands	47	55.29	1
	No response to verbal command	27	31.76	2
	Total	**85**	**100.00**	
Fasciculation	None	40	47.06	0
	Present - Generalised or continuous	33	38.82	1
	Both - Generalised and continuous	12	14.12	2
	Total	**85**	**100.00**	
Pupil size	> 2 mm	11	12.94	0
	< 2 mm	37	43.53	1
	Pin point	37	43.53	2
	Total	**85**	**100.00**	
Seizures	Present	5	5.88	1
	Absent	80	94.12	0
	Total	**85**	**100.00**	

The clinical presentation based on POP scoring system is depicted in the above table.

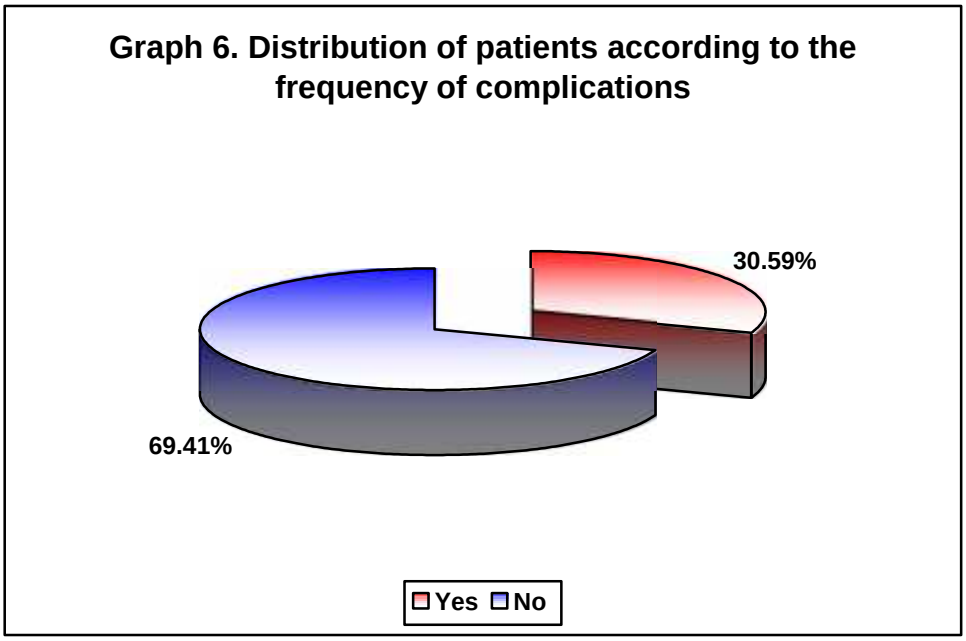
Table 7. Severity of intoxication based on POP score

Severity	POP score	Distribution (n=85)	
		Number	**Percentage**
Mild	≤ 3	26	30.59
Moderate	4 to 7	50	58.82
Severe	8 to 11	9	10.59
	Total	**85**	**100.00**

In our study we observed that 26 patients (30.59%) had mild intoxication, 50 patients (58.82%) had moderate intoxication and 9 patients (10.59%) had severe intoxication.

Table 8. Various complications

Complications (n=26)	Distribution	
	Number	Percentage
Acute renal failure	6	23.08
Intermediate syndrome	5	19.23
Respiratory failure	5	19.23
Cardiac arrhythmias	4	15.38
Pulmonary oedema	2	7.69
Optic neuropathy	1	3.85
Psychosis	1	3.85
Shock	1	3.85
Organophosphate induced delayed polyneuropathy	1	3.85
Total	**26**	**100.00**



A total of 26 patients had various complications, acute renal failure was the commonest complication observed in the study i.e. 6 patients (23.08%), intermediate syndrome 5 patients (19.23%), respiratory failure 5 patients (19.23%), cardiac arrhythmia 4 patients (15.38%) (2 ventricular tachycardia and 2 QTc prolongation). Other complications are shown in the above table.

Table 9. ECG findings

	Findings	Distribution (n=85)	
		Number	**Percentage**
At arrival	Normal	19	22.35
(Admission)	Sinus bradycardia	60	70.59
	Sinus tachycardia	6	7.06
	Total	**85**	**100.00**
During hospital	Sinus tachycardia	81	95.29
Stay	Ventricular tachycardia	2	2.35
	QTc prolongation	2	2.35
	Total	**85**	**100.00**

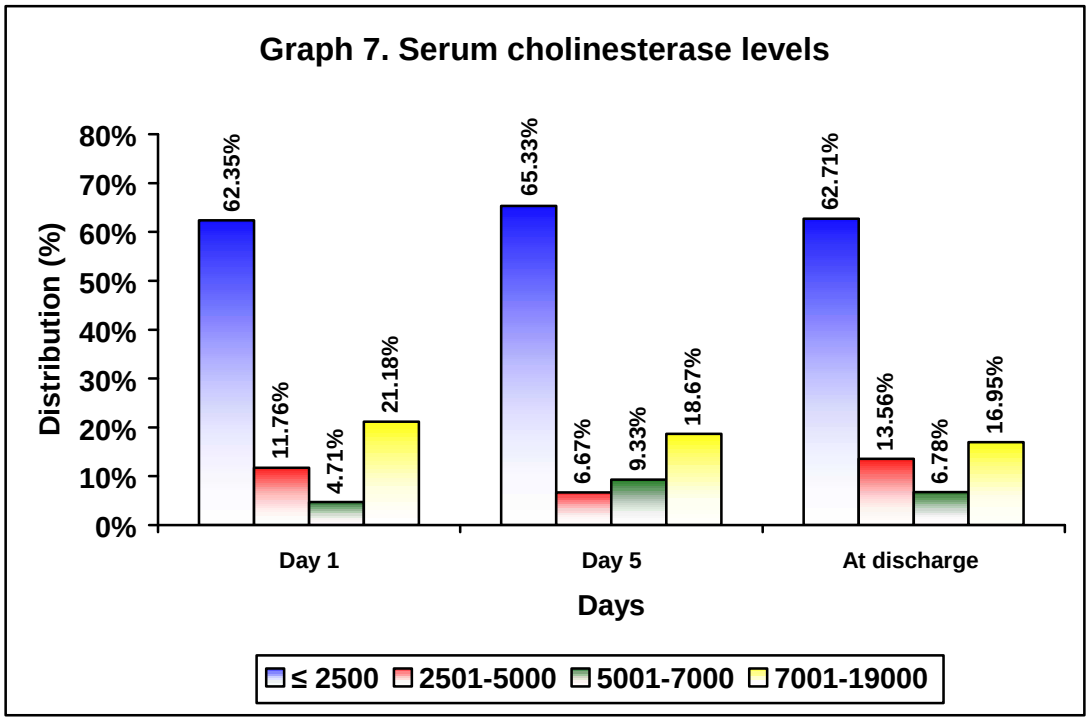
ECG tracing revealed 60 patients had sinus bradycardia at admission, 6 patients had sinus tachycardia and in remaining 19 patients it was normal.

During the hospital stay 81 patients had sinus tachycardia because of treatment with atropine, however 2 patients developed ventricular tachycardia and 2 had QTc prolongation.

LABORATORY PARAMETERS

Table 10. Levels of serum cholinesterase on Day1, Day5 and at discharge

Days	Serum cholinesterase levels (U/L)	Distribution	
		Number	Percentage
1st Day (n = 85)	≤ 2500	53	62.35
	2501-5000	10	11.76
	5001-7000	4	4.71
	7001-19000	18	21.18
	Total	**85**	**100.00**
5th Day(n = 75)	≤ 2500	49	65.33
	2501-5000	5	6.67
	5001-7000	7	9.33
	7001-19000	14	18.67
	Total	**75**	**100.00**
At discharge	≤ 2500	37	62.71
(n = 59)	2501-5000	8	13.56
	5001-7000	4	6.78
	7001-19000	10	16.95
	Total	**59**	**100.00**



In our study estimation of serum cholinesterase on Day 1 revealed 53 patients (62.35%) had ≤ 2500 Units/L, 18 patients (21.18%) in the range of 7001 – 19000, 10 patients (11.76%) in the range of 2501 – 5000 and only 4 patients (4.71%) in the range of 5001 – 7000. Similarly estimation of serum cholinesterase on 5th day and at discharge is shown in the above table.

Table 11. Renal function tests

Test	Findings	Distribution (n=85)	
		Number	**Percentage**
Blood Urea	Normal (14-36)	79	92.94
(mg/dL)	Elevated (>36)	6	7.06
	Total	**85**	**100.00**
Serum creatinine	Normal (0.80 – 1.30)	79	92.94
(mg/dL)	Elevated (>1.30)	6	7.06
	Total	**85**	**100.00**

In our study, 6 patients developed acute renal failure as a complication of organophosphorus compound poisoning and had elevated urea and creatinine, remaining patients had normal levels.

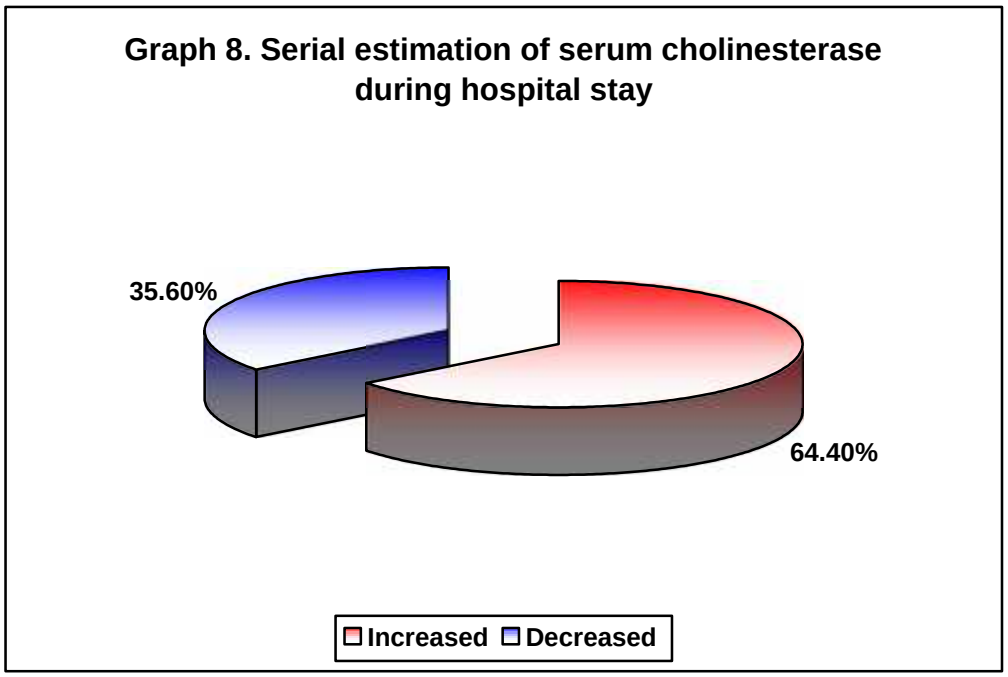
Table 12. Serum electrolytes

Electrolytes	Findings	Distribution (n=85)	
		Number	**Percentage**
Serum Sodium	Normal (130-145)	72	84.71
(mEq/L)	Elevated (>145)	10	11.76
	Low (<130)	3	3.53
	Total	**85**	**100.00**
Serum Potassium	Normal (3.0-5.0)	74	87.06
(mEq/L)	Elevated (>5.0)	3	3.53
	Low (<3.0)	8	9.41
	Total	**85**	**100.00**

Serum electrolyte levels of the 85 patients in our study have been depicted in the above table.

Table 13. Serial estimation of serum cholinesterase during hospital stay

Serum cholinesterase levels	Distribution (n=59)	
	Number	**Percentage**
Increased	38	64.40
Decreased	21	35.60
Total	**59**	**100**

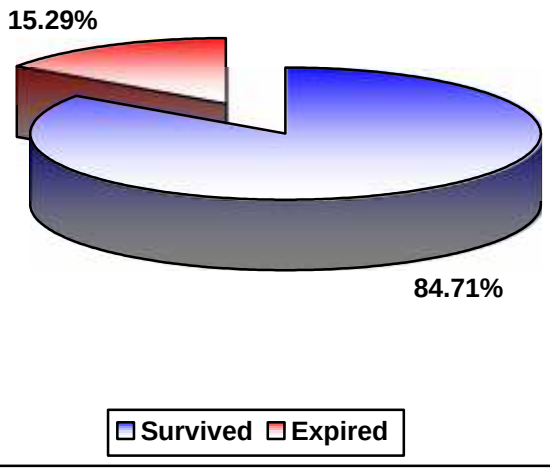


In our study we observed that 38 patients had an increasing trend of serum cholinesterase levels and 21 patients had a decreasing trend of serum cholinesterase levels, however in 26 patients it was not possible as some patients got discharged and some patients expired during serial estimation.

Table 14. Outcome of patients

Outcome	Distribution (n=85)	
	Number	**Percentage**
Survived	72	84.71
Expired	13	15.29
Total	**85**	**100.00**

Graph 9. Distribution of patients according to the outcome



In our study 72 patients (84.71%) survived and the remaining 13 patients (15.29%) expired.

Table 15. Death due to various causes

Cause	Distribution (n=13)	
	Number	**Percentage**
Intermediate syndrome	4	30.77
Respiratory failure	3	23.08
Pulmonary oedema	2	15.38
Acute renal failure	2	15.38
Cardiac arrhythmias	1	7.69
Shock	1	7.69
Total	**13**	**100.00**

We observed that out of 85 patients 13 expired (15.29%) who had different causes. Intermediate syndrome 4 patients (30.77%), Respiratory failure 3 patients (23.08%), Pulmonary oedema 2 patients (15.38%), Acute renal failure 2 patients (15.38%), 1 patient (7.69%) cardiac arrhythmia and 1 patient (7.69%) due to shock.

Table 16. Correlation of serum cholinesterase with severity of poisoning based on POP score

Serum cholinesterase levels (U/L)	Severity of poisoning (POP score) n=85					
	Mild		Moderate		Severe	
	No	%	No	%	No	%
≤ 2500	3	5.66	41	77.36	9	16.98
2501-5000	2	20.00	8	80.00	0	0.00
5001-7000	4	100.00	0	0.00	0	0.00
7001-19000	17	94.44	1	5.56	0	0.00
Total	**26**	**30.59**	**50**	**58.82**	**9**	**10.59**

p<0.001

In our present study correlation of serum cholinesterase with severity of poisoning based on POP score revealed, total of 50 patients had moderate intoxication, 26 patients had mild intoxication and 9 patients had severe intoxication as shown in the above table. p value<0.001 being statistically significant.

Table 17. Correlation of POP Scoring with hospital stay

Severity (POP score)	Hospital stay (Days)							
	< 3		4 to 7		8 to 14		> 14	
	No	%	No	%	No	%	No	%
Mild (<3)	8	30.77	16	61.54	1	3.85	1	3.85
Moderate (4-7)	1	2.00	9	18.00	33	66.00	7	14.00
Severe (8 - 11)	3	33.33	0	0.00	3	33.33	3	33.33
Total	**12**	**14.12**	**25**	**29.41**	**37**	**43.53**	**11**	**12.94**

p<0.001

In our study we correlated POP scoring with hospital stay as shown in the above table. p value <0.001 being statistically significant.

Table 18. Correlation of first day serum cholinesterase with hospital stay

Serum cholinesterase levels (U/L)	Hospital stay (Days)							
	< 3		4 to 7		8 to 14		> 14	
	No	%	No	%	No	%	No	%
≤ 2500	5	9.43	8	15.09	31	58.49	9	16.98
2501-5000	1	10.00	4	40.00	4	40.00	1	10.00
5001-7000	0	0.00	3	75.00	1	25.00	0	0.00
7001-19000	6	33.33	10	55.56	1	5.56	1	5.56
Total	**12**	**14.12**	**25**	**29.41**	**37**	**43.53**	**11**	**12.94**

p<0.001

In our present study we correlated first day serum cholinesterase with days of hospital stay as depicted in the above table. p value < 0.001 being statistically significant.

Table 19. Correlation of serial estimation of serum cholinesterase with hospital stay

	Serum cholinesterase levels (U/L)					
	Increased			Decreased		
	n	Mean	SD	n	Mean	SD
Hospital stay(Days)	38	9.40	3.84	21	11.50	3.51

p=0.043

Attempt to compare mean hospital stay with serial estimation of serum cholinesterase showed stay of patients being 11.50 ± 3.51 days in patients with decreasing trend of serum cholinesterase as compared to 9.40 ± 3.84 days in patients with increasing trend. p value = 0.043 being statistically significant.

Table 20. Correlation of first day serum cholinesterase with complications

Serum cholinesterase levels (U/L)	Complications			
	Without		With	
	Number	Percentage	Number	Percentage
≤ 2500	33	62.26	20	37.74
2501-5000	7	70.00	3	30.00
5001-7000	3	75.00	1	25.00
7001-19000	16	88.89	2	11.11
Total	**59**	**69.41**	**26**	**30.59**

p=0.183

The above table depicts the percentage of complications with various levels of cholinesterase done on day 1. p value = 0.183 being statistically insignificant.

Table 21. Correlation of first day serum cholinesterase with ventilatory support

Serum cholinesterase levels (U/L)	Ventilatory support (n=85)			
	Without		With	
	Number	Percentage	Number	Percentage
≤ 2500	35	66.04	18	33.96
2501-5000	7	70.00	3	30.00
5001-7000	4	100.00	0	0.00
7001-19000	17	94.44	1	5.56
Total	**63**	**74.12**	**22**	**25.88**

p=0.047

In our present study of 85 patients 63 patients did not require ventilatory support, remaining 22 patients required ventilatory support for various reasons. (6 patients of acute renal failure, 5 patients of intermediate syndrome, 5 patients of respiratory failure, 3 patients of cardiac arrhythmia, 2 patients of pulmonary oedema and 1 patient with shock) p value = 0.047 being statistically significant.

Table 22. Correlation of serial estimation of serum cholinesterase with ventilatory support.

Serum cholinesterase levels	Ventilatory support			
	Without		With	
	Number	Percentage	Number	Percentage
Increased	31	81.58	7	18.42
Decreased	13	61.90	8	38.10
Total	**44**	**74.58**	**15**	**25.42**

p=0.097

Correlation of serial estimation of serum cholinesterase with ventilatory support revealed that a higher percentage of patients with decreasing trend of serum cholinesterase levels required ventilatory support than the patients with increasing trend as shown in the above table. p value = 0.097 being statistically insignificant.

Table 23. Correlation of first day serum cholinesterase with outcome

Serum cholinesterase levels (U/L)	Outcome			
	Survived		Expired	
	Number	Percentage	Number	Percentage
≤ 2500	42	79.25	11	20.75
2501-5000	9	90.00	1	10.00
5001-7000	4	100.00	0	0.00
7001-19000	17	94.44	1	5.56
Total	**72**	**84.71**	**13**	**15.29**

p=0.451

In our study first day estimation of serum cholinesterase was correlated with overall outcome of patients which is shown in the above table. p value = 0.451 being statistically insignificant.

Table 24. Correlation of serial estimation of serum cholinesterase with outcome of patients

Serum cholinesterase levels	Outcome			
	Survived		Expired	
	Number	Percentage	Number	Percentage
Increased	38	100.00	0	0.00
Decreased	16	76.19	5	23.81
Total	**54**	**91.53**	**5**	**8.47**

p=0.004

In our present study out of 59 patients 38 patients had increasing trend of serum cholinesterase levels and 21 patients had decreasing trend of cholinesterase levels. In patients with increasing trend of serum cholinesterase there was no death observed and in decreasing trend 5 patients expired. p value = 0.004 being statistically significant.

DISCUSSION

In the present study of 85 patients with organophosphorus compound poisoning a correlation between serum cholinesterase levels and clinical outcome was studied and same was compared with the POP scoring system and other variables.

All the 85 patients who presented with organophosphorus compound poisoning were with a suicidal bid. This is similar to a study done by Khazi MA et al.[99]

In our study patient age ranged from 18 to 87 years. Maximum number of cases were in the age group of below 30 years i.e. 47 patients (55.29%). This is similar to study done by Sen R et al,[100] Rehiman et al.[94] and Patil G et al.[101]

When sex was taken into consideration we observed 60 males and 25 females were present in the study group. There was male preponderance with a ratio of (male to female 2.4:1). This observation is similar to study by Kang EJ et al.[19] and Patil G et al.[102] In contrast Rehiman et al.[94] and Sen R et al.[100] observed more number of females in their study.

We tried to analyse our patients with the organophosphorus compounds they had consumed which is shown in table 3. The commonly consumed compounds were malathion 21 patients (24.71%), chlorpyrifos 14 patients (16.47%) and parathion 14 patients (16.47%). In studies by Nouira et al.[97] and Rehiman et al.[94] the most commonly consumed compounds were parathion and dichlorvos. In contrast a

study by Kumar CU et al.[91] found that the most commonly consumed compounds were monocrotophos and chlorpyrifos.

Similarly we tried to analyse the time of consumption to arrival at the hospital. We had 53 patients (62.35%) who presented after 3 to 6 hours of consumption, 31 patients (36.47%) within 3 hours and only 1 patient after 6 hours. Nouira et al.[97] observed that in their study patients presented within a mean time interval of 2.5 hours (range, 30 minutes to 15.5 hours).

Patients presented with various symptoms of organophosphorus compound poisoning. Majority had vomiting 74 patients (87.06%), sweating 60 patients (70.59%), breathlessness 57 patients (67.06%), other symptoms were diarrhoea 53 patients (62.35%), muscle twitching 46 patients (54.12%) and only 2 patients presented with seizures. Similarly studies by Nouira et al.[97] and Venkateshwarlu N et al.[102] observed combination and permutation of symptoms of nausea, vomiting, fasciculations, diarrhoea, etc.

An attempt to study clinical presentation based on POP score revealed severe intoxication in 9 patients (10.59%), moderate intoxication in 50 patients (58.82%) and mild intoxication in 26 patients (30.59%). Same is depicted in table 6 (POP score) and table 7 (severity). This is in contrast with study by Rehiman et al.[94] who observed 70% of their cases had mild intoxication, 26% cases had moderate intoxication and only 4% cases had severe intoxication.

We observed various complications of organophosphorus compound poisoning 6 patients (23.08%) had acute renal failure, intermediate syndrome 5 patients (19.23%), respiratory failure 5 patients (19.23%), cardiac arrhythmia

4 patients (15.38%) (2 ventricular tachycardia and 2 QTc prolongation). Other complications are depicted in table 8. This is in contrast to a study by Venkateshwarlu N et al.[103] who observed that, pulmonary oedema was the most common complication seen in their patients. Sen R et al.[100] found respiratory acidosis to be the most common complication followed by intermediate syndrome.

In our study all 85 patients were subjected to ECG tracing at arrival and during their stay in the hospital which is shown in table 9. Yun et al.[93] had made an attempt to study QTc prolongation and its correlation with mortality in their patients. A statistically insignificant difference was found between the mean QTc intervals of the patients who survived and those who expired.

An attempt was made to study various lab parameters in our patients. Serum cholinesterase estimation done on serial days (i.e. day1, day5 and at discharge) revealed various levels on different days which is depicted in table 10. A study by Prasad DRMM et al.[12] revealed similar observations.

Taking renal functions into consideration, all 6 patients who developed acute renal failure had elevated urea and creatinine levels. Remaining 79 patients had normal urea and creatinine levels. 3 patients with acute renal failure were subjected to hemodialysis and 3 were not. One patient expired in each group. Many of our patients had deranged serum electrolytes as depicted in table 12.

Kang EJ et al.[19] had subjected their patients for hemoperfusion and found that it was ineffective in terms of survival and improvement of patients with organophosphorus compound poisoning. Study by Altintop et al.[103] reported hemoperfusion to be useful in severe cases. This is by a proposed mechanism that

activated charcoal or resins used for hemoperfusion may help in purifying the blood thus allowing the patients to improve. We did not attempt hemoperfusion in any of our patients since there was no definite evidence of benefit in terms of morbidity or mortality.

We subjected our patients to liver function tests and all of them presented with grossly normal serum bilirubin, SGOT, SGPT and alkaline phosphatase. In our study we have safely avoided patients with known liver disease. (Chronic liver disease is known to affect serum cholinesterase levels). Patil G et al.[101] have also excluded patients with known liver disease in their study.

Serial estimation of serum cholinesterase levels (day1, day5 and at discharge) in 59 patients revealed 38 patients (64.40%) had an increasing trend and 21 patients (35.60%) had a decreasing trend. In the remaining 26 patients it was not possible to study the trend as some got discharged and some expired during serial estimation. Studies by Kumar CU et al.[91] and Yun et al.[93] have also attempted similar estimation and analysis of serum cholinesterase levels.

In our study the outcome of patients was evaluated and we found that 13 patients (15.29%) had expired and 72 patients (84.71%) had survived. A study by Kang EJ et al.[19] found almost similar outcomes of patients. In contrast, Siva Prabodh V. et al.[2] had observed a significantly higher percentage of deaths in their study.

An attempt to find out the various causes of death in our patients revealed Intermediate syndrome 4 patients (30.77%), Respiratory failure 3 patients (23.08%), Pulmonary oedema 2 patients (15.38%), Acute renal failure 2 patients (15.38%),

1 patient (7.69%) cardiac arrhythmia and 1 patient (7.69%) due to shock. Studies[12,94-102] by different authors have not commented on the causes of death.

In our study a statistically significant correlation was found between serum cholinesterase levels and severity of poisoning based on POP score. ($p<0.001$) This is similar to a study done by Khazi MA et al.[99] Another study by Sen R et al.[100] also reported significant correlation but in their study comparison was done not only with serum cholinesterase, other parameters like CPK and LDH were also taken into account.

An attempt to correlate POP scoring with hospital stay of patients showed a significant correlation as shown in table 17. ($p<0.001$; statistically significant). Similar observations were made by Rehiman et al.[94] and Manu et al.[88]

We observed that first day serum cholinesterase was directly correlating with hospital stay which is depicted in table 18. ($p<0.001$; statistically significant) Similar observations were made by Khazi MA et al.[99] and Rehiman et al.[94] wherein a significant correlation was established between deranged serum cholinesterase level and morbidity of the patients in terms of prolonged duration of hospital stay.

We also attempted a correlation of serum cholinesterase estimation on serial days which showed a significant correlation of serum cholinesterase with hospital stay. Mean hospital stay was 9.40±3.84 days in patients with an increasing trend of serum cholinesterase levels whereas in patients with a decreasing trend the mean hospital stay was 11.50±3.51 days. ($p=0.043$; statistically significant)

When we attempted to correlate levels of serum cholinesterase on first day with complications, we found that there were more complications in patients whose

levels were ≤2500 U/L i.e. 20 patients (37.74%) as well as in those with levels between 2501 to 5000 U/L i.e. 3 patients (30.00%). However p value = 0.183 was statistically insignificant. Different authors have not correlated levels of serum cholinesterase with complications.

A comparison between first day serum cholinesterase estimation and ventilatory support is depicted in table 21. Out of the 53 patients with serum cholinesterase levels ≤2500 U/L 18 patients (33.96%) required ventilatory support. Similarly 3 patients (30.00%) with a serum cholinesterase of 2501-5000 U/L and only one patient (5.56%) with serum cholinesterase of 7001 – 19000 required ventilatory support. (p=0.047; statistically significant). Studies by Khazi MA et al,[99] Kumar CU et al.[91] and Prasad DRMM et al.[12] have also found correlation between first day serum cholinesterase estimation and ventilatory support.

Correlation of serial estimation of serum cholinesterase with ventilatory support showed that 8 patients (38.10%) with a decreasing trend of serum cholinesterase levels required ventilatory support as opposed to 7 patients (18.42%) with an increasing trend. However it did not have a significant p value (p=0.097) A similar study by Goswamy et al.[20] found significant correlation between serial estimation of serum cholinesterase and patients requiring ventilatory support. They found that low serum cholinesterase levels have greatest predictive value for mechanical ventilation. A study by Nouira et al.[97] did not find any correlation between low levels of serum cholinesterase and requirement of ventilatory support.

Similarly we tried to correlate first day serum cholinesterase estimation with outcome which is depicted in table 23. (p=0.451; statistically insignificant). In a

study by Rehiman et al.[94] it was observed that serum cholinesterase levels at presentation did not correlate with mortality. In contrast studies by Kumar CU et al,[91] Patil G et al.[101] and Prasad DRMM et al.[12] have found a significant correlation between first day serum cholinesterase and mortality.

Finally, correlation of serial estimation of serum cholinesterase with outcome of patients was done. In patients with an increasing trend of serum cholinesterase levels 38 patients (100%), all survived and none expired whereas in patients with a decreasing trend 16 patients (76.19%) survived and 5 patients (23.81%) expired. ($p=0.004$; statistically significant). Similarly Yun et al.[93] and Chen et al.[104] noted a statistically significant relationship between dynamic changes of serum cholinesterase activity and mortality.

In our present study with a small sample size of 85 patients we observed a significant correlation of serum cholinesterase when compared to hospital stay of patients (estimation at arrival and serial estimation). When compared to ventilated patients the correlation was also significant (first day but not on serial estimation). Taking overall outcome into account it was found to be significant on serial estimation of serum cholinesterase (but not on first day estimation).

A few studies have shown lack of relationship between serum cholinesterase activity and clinical outcome. Serum cholinesterase may not be a reliable biomarker in assessing the clinical severity of organophosphorus compound poisoning. This is proposed by a mechanism that tissue concentration of cholinesterase (true cholinesterase), which is thought to be more closely related to clinical manifestations of organophosphorus poisoning is poorly correlated with plasma

level of enzyme (pseudocholinesterase / serum cholinesterase). The sensitivity of these two enzymes (true cholinesterase and pseudocholinesterase) to organophosphorus inhibition may be different. And it may be possible that some of the organophosphorus compound's toxic manifestations are independent of cholinergic mediated mechanism.

So we feel it is prudent to study with a large sample size and estimation of true cholinesterase enzyme levels and to find other mechanisms of toxicity which are independent of enzyme inhibition.

CONCLUSION

In our present study of 85 patients with organophosphorus compound poisoning we observed various clinical manifestations and same were compared with the POP scoring system. Levels of serum cholinesterase were correlated with various variables such as hospital stay, complications, ventilatory support and outcome.

We found statistically significant correlation between serum cholinesterase and hospital stay in days (first day levels and serial estimation); serum cholinesterase and requirement of ventilatory support (first day levels alone); serum cholinesterase and outcome (serial estimation alone). Similarly the correlation between serum cholinesterase and complications was not statistically significant.

We feel it is worth to study by adjusting other variables like age, sex, time of consumption to arrival at the hospital, quantity and toxicity of different compounds.

Owing to our small sample size (85 patients), a larger sample size may be required to address these issues.

SUMMARY

The present study of 85 patients with organophosphorus compound poisoning admitted in department of medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the study period from January 2014 to December 2014, was undertaken to correlate serum cholinesterase and clinical outcome.

The results observed were significant correlation of serum cholinesterase and hospital stay in days (first day levels and serial estimation); serum cholinesterase and requirement of ventilatory support (first day levels); serum cholinesterase and outcome (serial estimation).

However we did not find significant correlation with variable factors – age, sex, type of compound, quantity and time interval from consumption to arrival at the hospital.

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ANNEXURE I – CONSENT FORM

TITLE OF RESEARCH STUDY: ASSOCIATION BETWEEN SERUM CHOLINESTERASE LEVELS AND CLINICAL OUTCOME IN PATIENTS OF ORGANOPHOSPHORUS COMPOUND POISONING

Principal Investigator:
Dr. *** *******
Post Graduate Student,
Department of General Medicine,
Jawaharlal Nehru Medical College,
Belgaum – 590 010

Guide:
Dr. **** ********
Professor & Head of Unit,
Department of General Medicine,
Jawaharlal Nehru Medical College,
Belgaum – 590 010

Introduction and Purpose

Organophosphorus compound poisoning is one of the commonest poisonings seen in clinical practice. This study intends to demonstrate the value of serum cholinesterase levels as a prognostic marker for patients with organophosphorus poisoning to assess the level of morbidity of the patient and severity of symptoms.

Procedure

If you agree to be part of the research study, you will be asked the relevant history and will be subjected to relevant clinical examination and investigations. You will also have to give blood and urine samples for the necessary investigations.

Risk and Benefits

The only risk and possible discomfort you might get is while taking blood from your arm for the investigations. It may cause swelling, pain, redness (rarely happens) at the site from where the blood is drawn.

You may not be benefitted by these investigations but you will be part of this study which is going to be useful to others in the future.

Alternatives

Taking part in this study is voluntary. You may choose not to take part in this study.

If you decide to take part you can later change your mind and withdraw from the study. Your decision will not change the present or future health care or other services that you receive. The study doctor or sponsor may stop your participation in this study at any time. If you choose not to take part in the study, you will receive the standard treatment for patients with your condition.

Privacy and Confidentiality

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study may be published but your identity will be confidential in any publication.

Institution / Sponsor's policy

Does not apply to this research

Financial incentives for participation

You will not be paid / offered any gifts / incentives for participating in the study.

Authorization to publish the results

The results of the study would be forwarded to the KLE University, Belgaum as part of requirement towards the completion of MD degree, review and publishing.

In case of the queries during study or in future you may contact following persons,

1. Dr. *** *******,**
Chairman,
Jawaharlal Nehru Medical College,
Ethical Committee for Human Research
Phone Number - ***** *****

2. Dr. *** **********
**
Professor & Head of Unit,
Dept of General Medicine,
Jawaharlal Nehru Medical College,
Belgaum – 590 010
Phone Number – ***** *****

3. Dr. **** ******
**
Investigator,
PG in General Medicine,
Jawaharlal Nehru Medical College,
Belgaum – 590 010
Phone Number – ***** *****

CONSENT FORM

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicates that I have read this consent form, or it has been read to me and has been explained to me in my vernacular language and all my questions have been answered. I will be given a copy of this consent form.

Signature / Left Thumb print of the Participant or legally authorized representative:

Participant Name: ……

Signature / Left thumb impression of the participant: ……..

Name of the legally authorized representative / guardian: ……

Signature / Left thumb impression: ……...

Witness Name: ----------

Signature / Left thumb impression: ……...

Investigator Name and signature: ……...

Date:

Place:

ANNEXURE II – PROFORMA

TITLE: ASSOCIATION BETWEEN SERUM CHOLINESTERASE LEVELS AND CLINICAL OUTCOME IN PATIENTS OF ORGANOPHOSPHORUS COMPOUND POISONING

Case No. :

Name :

Age / sex :

Inpatient Number :

Address :

Occupation :

History

1. Informant Patient / Relative / Others

2. Type of OP compound consumed

3. History of consumption of Alcohol/ Kerosene/ Any other poison

4. Route of exposure

5. Quantity consumed

6. Time interval from ingestion to hospitalization

7. Intention Homicidal / Suicidal / Accidental

Complaints at presentation	Yes	No
Vomiting		
Diarrhea		
Sweating		
Breathlessness		
Muscle twitching		
Convulsions		

Any other complaint

Past history	Yes	No
Consumption of poison		
Psychiatric illness		
Ischemic heart disease		
Diabetes mellitus		
Hypertension		
Any other		

Family history

Personal history

Treatment history

Physical examination

Temperature :

Pulse :

Respiratory rate :

Blood pressure :

Pupils :

Systemic examination

Respiratory system :

Cardiovascular system :

Per abdomen :

Central nervous system :

POP score

Diagnosis

Duration of stay in hospital

Complications

Requirement of mechanical ventilation Yes/No

If Yes, duration of mechanical ventilation

Outcome Survived / Expired

If expired, cause of death

ANNEXURE III – KEY TO MASTER CHART

-	-	Absent
/cumm	-	Per cubic millimeters
+	-	Present
^{0}F	-	Degree Fahrenheit
A	-	Absent
ARF	-	Acute renal failure
BP	-	Blood pressure
Br	-	Bradycardia
C	-	Chlorpyrifos
CA	-	Cardiac arrhythmia
CNS	-	Central nervous system
DLC	-	Differential leukocyte count
DM	-	Dimethoate
DV	-	Dichlorvos
DZ	-	Diazinon
E	-	Expired
ECG	-	Electrocardiogram
F	-	Female
f	-	Fenthion
FC	-	Fenchlorophos
gm	-	Gram
IS	-	Intermediate syndrome
m	-	Malathion

M	-	Male
MC	-	Monocrotophos
mEq/L	-	Milliequivalents per liter
mg/dL	-	Milligrams per deciliter
mm Hg	-	Millimeters of mercury
MP	-	Methylparathion
n	-	No
N	-	Normal
OIDP	-	Organophosphate induced delayed polyneuropathy
ON	-	Optic neuropathy
P	-	Present
PO	-	Pulmonary oedema (Non cardiogenic)
POP	-	Peradenya Organophosphorus Poisoning
Pr	-	Parathion
QTcP	-	QTc prolongation
RF	-	Respiratory failure
s	-	Suicidal
S	-	Survived
SB	-	Sinus bradycardia
SGOT	-	Serum glutamic oxaloacetic transaminase
SGPT	-	Serum glutamic pyruvic transaminase
ST	-	Sinus tachycardia
T	-	Tachycardia
Tc	-	Tachypnoea
TLC	-	Total leukocyte count

U	-	Unknown
U/L	-	units per liter
VT	-	Ventricular tachycardia
Y	-	Yes