"SERUM CHOLINESTERASE LEVELS IN ORGANOPHOSPHORUS POISONING PATIENTS ON VENTILATORY SUPPORT"

Submitted by

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Dissertation submitted to the

B. L. D. E. U'S SHRI B. M. PATIL MEDICAL COLLEGE AND RESEARCH CENTRE, VIJAYAPUR. KARNATAKA

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MD

IN

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Under the guidance of

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LIST OF ABBREVIATIONS

Ach	-	Acetycholine
AchE	-	Acetyl cholinesterase
CoA	-	Coenzyme A
CPAP	-	Continuous Positive Airway Pressure
CSF	-	Cerebrospinal fluid
DM	-	Diabetes Mellitus
D/D	-	Discharge/death
EDTA	-	Ethylenediaminetetraacetic acid
FiO2	-	Fraction of inspired Oxygen
H/O	-	History of
ICU	-	Intensive Care Unit
I:E ratio	-	Inspiratory to Expiratory time ratio
IM	-	Intramuscular
IU/L	-	International units per litre
IV	-	Intravenous
N	-	Number
OP	-	Organophosphate
PAM	-	Pralidoxime
PEEP	-	Positive end expiratory pressure
PS	-	Pressure Support
SCE	-	Serum Cholinesterase
SIMV	-	Synchronized Intermittent Mandatory Ventilation
SpO2	-	Oxygen saturation
WHO	-	World Health Organisation

ABSTRACT

Background-

Organo-phosphorus poisoning is a major public health problem in developing world. OP poisoning is associated with decrease in serum cholinesterase level. This study is aimed to estimate serum cholinesterase enzyme levels serially and to evaluate the prognostic value of serum cholinesterase in OP poisoning patients.

Materials and Methods-

This is a prospective study done at Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapur with a sample size of 70. Serial estimation of serum cholinesterase was done at the time of admission, 3^{rd}, 5^{th}, 7^{th},9^{th}, and 11^{th} day in patients who fulfilled the inclusion criteria.

Results-

There were 42 males and 28 females. Majority of them (73%) were below 40years of age, and from rural areas. There was suppression of SCE in all of the patients on the day of presentation. 55 out of 70 patients had values of < 1962 IU/L. Majority (>78%), had moderate to severe poisoning based on serum cholinesterase values, as per Proudfoot classification. Only 21% had mild poisoning.

Conclusion-

Study revealed decrease levels of pseudocholinesterase levels at the time of admission in organophosphorus poisoning patients with subsequent return to normal values during hospital stay. Their was direct correlation between the severe suppression of SCE levels and the clinical severity of poisoning such as presence of ventilator insufficiency and mortality. There was good correlation between improvement in patient's motor power and improved serum cholinesterase levels during the time the patients were successfully weaned.

KEYWORDS- organophosphate poisoning, plasma cholinesterase.

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INTRODUCTION

Poisoning is one of the commonest causes of admission of young adults in the intensive care units in india[1]. Pesticide poisoning is a major public health problem in developing world. Millions of people are exposed to danger of hazardous occupational practices and unsafe storage of pesticides[2]. Organophosphorus pesticides poisoning can result from occupational, accidental or intentional exposure. However, it is deliberate self-poisoning which causes the great majority of deaths and places immense strain on hospital services. According to a World Health Organization (WHO) report, three million cases of pesticide poisoning occur annually worldwide and most of them are in Asia of which at least half of them are due to organophosphorus (OP) poisoning.[2,3] They are estimated to cause 220,000 fatalities annually[3].

OP compounds are amongst the most common poisons used for deliberate self-poisoning in India and other parts of the world[4]. In many reports from India, rate of suicidal poisoning with OP compounds ranges from 10.3 to 43.8%[5]. Among OP poisoned patients in India, hospital mortality rate is reported to be as high as 20-70%[6]. The exact incidence of organophosphorus poisoning in India is uncertain due to lack of data or lack of proper reporting. Being predominantly an agricultural country, pesticides and insecticides are used abundantly for cultivation and access to these poisonous chemical substances by the population is easy. Among adults, incidence is more in females of all age groups and generally, those in second and third decades of life are more likely to be affected. In 1854 Clermont published the synthesis of highly potent anti-cholinesterase compound, tetraethyl pyrophosphate (TEPP)[7]. In 1914, Dall postulated the presence of enzyme, esterase in blood which was later established by Nauratal and Plattner. In 1930 Stedman named it cholinesterase[8]. Modern

investigations of organophosphorus compounds date from 1932 with publication of Lange and Krueger on synthesis of dimethyl and diethyl phosphoro fluoridate. In 1955, Wilson and Ginsberg introduced 2- pyridine aldoxime methochloride (2-PAM) as a reactivator of organophosphate inhibited cholinesterase[9].

Organophosphorus compounds are anti acetylcholinesterases which exert their toxicity by interfering with the normal function of acetylcholine, an essential neuro - transmitter throughout the autonomic and central nervous system. The manifestations of toxicity are a result of this effect, affecting the patient's physiology.

There are two forms of cholinesterases

1. True cholinesterase or acetyl cholinesterase, is located in erythrocytes, neuromuscular junctions and gray matter of brain.
2. Pseudocholinesterase, serum or plasma cholinesterase, is primarily present in serum but is also present in liver, pancreas and heart[10].

Both these types of enzymes are inhibited by insecticide poisoning.

Previous studies associating the severity or prognosis of Organophosphorus poisoning with estimation of serum cholinesterase have been contradictory. *Goswamy R. et al*[11]. concluded that apart from clinical indicators, low serum cholinesterase levels were of greatest predictive value for ventilation in organophosphorus poisoning. However, *Aygun D et al.*[12] found that serum cholinesterase level estimations are useful in diagnosis of organophosphorus poisoning in acute phase but show no relation to severity of poisoning.

The present study aims to correlate serum cholinesterase levels; with clinical course and to predict the clinical outcome of organophosphorus poisoning.

AIMS AND OBJECTIVES

1. To estimate Serum Cholinesterase enzyme levels serially in acute Organophosphorus poisoning.
2. To correlate the Serum Cholinesterase levels to the severity of Organophosphorus poisoning and outcome.

ANATOMY & PHYSIOLOGY OF AUTONOMIC NERVOUS SYSTEM

The portion of the nervous system which controls the visceral functions of the body is called autonomic nervous system. This helps in control of arterial pressure, gastro-intestinal motility, secretions, urinary bladder control, sweating and body temperature etc. One of the most striking characteristics of the autonomic nervous system is the rapidity and intensity with which it can change visceral function.

Autonomic nervous system centers are located in the hypothalamus, brain stem and spinal cord.

SYMPATHETIC:

Spinal cord - T1 – L1

Pre - vertebral ganglia - coeliac and hypogastric

PARASYMPATHETIC:

From central nervous system – III, VIII, IX, X cranial nerves.

From spinal cord – S2, S3, S4 nerves.[13]

ANATOMY & PHYSIOLOGY OF N.M. JUNCTION

ACETYLCHOLINE:

Acetylcholine (Ach), first synthesized by BAYER in 1867, is a neurotransmitter. It was first recognized as a potent pharmacological substance by HUNT in 1906.

Acetylcholine is produced at

a) Autonomic effector sites innervated by postganglionic parasympathetic fibres.

b) Preganglionic autonomic fibres of sympathetic and parasympathetic ganglion cells and adrenal medulla.

c) Motor end-plates on skeletal muscle

d) Certain synapses in central nervous system

The Ach in the motor nerve terminal is synthesized in the axoplasm from choline and Acetyl CoA by a process facilitated by the enzyme choline acetyl transferase. The choline necessary for this is derived from extra cellular fluid which is transported into the nerve terminal by a carrier mediated transport system.[13]

About 20% of Ach in nerve terminal is present as free Ach in the axoplasm, and 80% is contained within the vesicles, each containing about 4-5 x 10^5 molecules of Ach.

Seperate pools or stores of Ach exist within the nerve terminal. Most of the Ach (80%) can be released by nerve impulses (the releasable pool), but some cannot (the non-releasable pool or stationary pool). The releasable pool consists of the Ach contained within the vesicles, whereas non-releasable pool is the Ach of the axoplasm. Releasable pool is often divided into immediately available and the reserve pool.

Acetylcholine acts through two receptors:

MUSCARINIC RECEPTORS

Muscarine is a poison from toad stools that activates only muscarinic receptors. Effector cells are stimulated by postganglionic neurons of the parasympathetic nervous system and also postganglionic cholinergic neurons of the sympathetic nervous system.

NICOTINIC RECEPTORS

Nicotine will activate the nicotinic receptors in pre and post ganglionic neurons of both the sympathetic and parasympathetic systems and also in the membranes of skeletal muscle fibres at neuromuscular junction.[13]

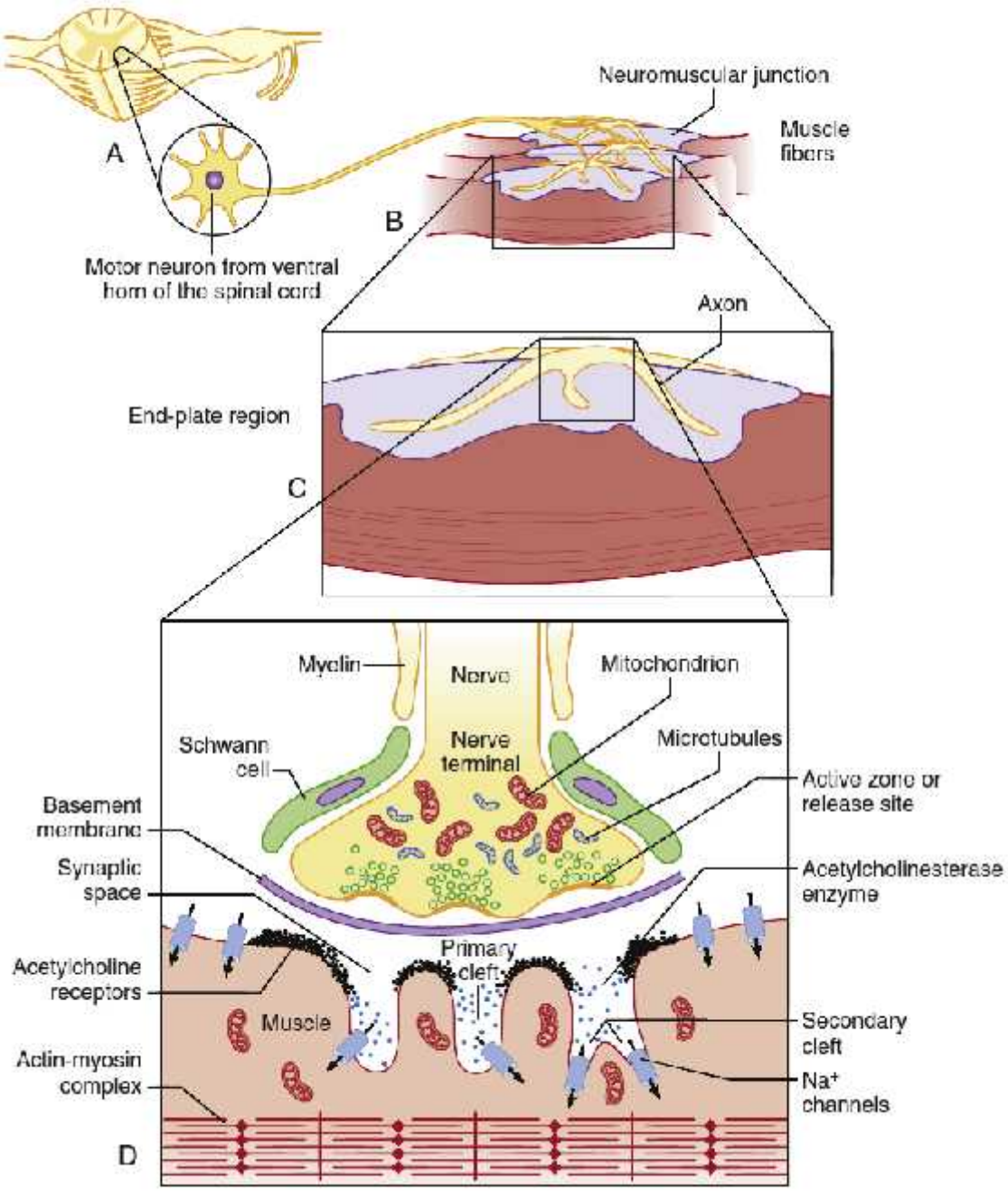


Figure No. 1 : Anatomy of Neuromuscular Junction – *The Motor End Plate*

Metabolism of Acetyl choline:

Junctional acetylcholinesterase is the enzyme responsible for the hydrolysis of Ach in the synaptic cleft.

Acetyl cholinesterase is a protein attached to the basement membrane of the muscle and probably also to membranes of the motor end plates and the nerve terminals. Each molecule of the enzyme is able to bind and hydrolyze several molecules of acetylcholine. It has been estimated that for each molecule of Ach released by a nerve impulse, there are atleast 10 active enzymes sites available. This arrangement ensures that each Ach molecule only reacts once with the receptor, after which it is rapidly (in < 1msec) hydrolysed.[14]

TYPES OF CHOLINESTERASE: Two major forms of cholinesterase exist in vertebrates which hydrolyze acetyl choline[15]

PLASMA CHOLINESTERASE: (Pseudo- or Butyryl Cholinesterase)

It is found in plasma, liver, pancreas and intestinal mucosa. (Liver being the main organ). Variations occur due to liver disease, chronic inflammation, malnutrition, morphine, codeine, succinylcholine administration and hypersensitivity reactions.

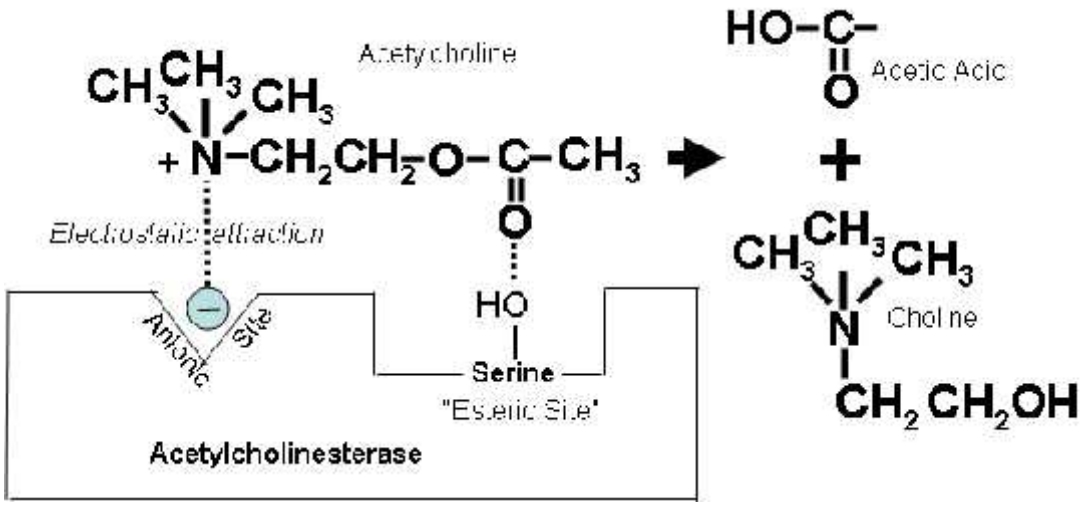
RBC CHOLINESTERASE: (True, Specific Cholinesterase)

It is found in nervous tissue, erythrocytes, lung, spleen and grey matter. It is decreased in pernicious anemia and after anti-malarial therapy.

Acetylcholine is inactivated by combination with two sites on the enzyme RBC cholinesterase: Anionic site and Esteratic site

ANIONIC SITE:- Bears a negative charge which attracts the quaternary nitrogen ion (N^+) of acetylcholine.

ESTERATIC SITE:- Attracts the carboxyl group of Acetylcholine molecule and the esteratic site of the enzyme is acetylated and this results in splitting of choline. The acetyl group in combination with the esteratic site is however immediately removed as a result of combination with water, forming acetic acid. This sets the esteratic site of the enzyme free, for further inactivation of acetylcholine.[16]



It is the esteratic site (shown above) of the acetylcholinesterase that the organophosphate compounds bind irreversibly to form phosphorylated enzyme.

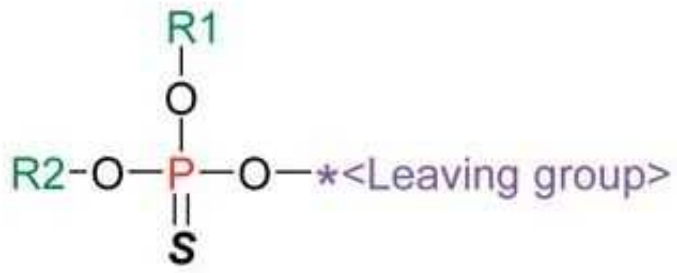
PHARMACOLOGY OF ORGANO PHOSPHORUS COMPOUNDS

The Organophosphorus compounds are IRREVERSIBLE ANTICHOLINESTERASES which combine with the enzyme cholinesterase and make them inactive. These combine with only esteratic site of cholinesterase and consequently esteratic site is phosphorylated. The hydrolysis of phosphorylated site is however slow.

This inhibition of acetylcholinesterase causes acetylcholine to accumulate at synaptic cleft. The Ach then acts at the cholinergic receptor sites and thus, potentially capable of producing effects equivalent to excess stimulation of cholinergic receptors throughout the central and peripheral nervous system.[16]

Structure of Organophosphorus compounds

O.P. compounds are usually esters, amides or thiol derivatives of phosphoric or phosphonic acids.



General chemical structure of an organophosphate

R_1 and R_2 are usually simple alkyl or aryl groups, X is referred to as the "leaving group", maybe heterocyclic groups linked to -O- or -S-. The double bonded atom may be O or S and the related compound is hence termed a phosphate or phosphorothioate.

The P=S form is intrinsically more stable and many insecticides are manufactured in this form.

Oxidation of phosphorothioates to phosphates is potentially dangerous, as phosphates are more volatile and directly toxic.

Commercial formulations of organophosphorus insecticides may contain more than one organophosphorus compound.

The biological behaviour and toxicity to man of organophosphorus compound combinations and impurities may be different from those of the single compound.

PHARMACOKINETICS

Most organophosphorus compounds are rapidly and well absorbed from the skin , mucous membrane, conjunctiva, gastro-intestinal tract and lungs. These chemicals are detoxified by cytochrome P450 mediated mono-oxygenases in liver. But some metabolites are more toxic than parent compounds as the case in the conversion of parathion, diazinon and malathion to oxons.[17]

Classification of Insecticides:[15]

OrganoChlorine Compounds	OrganoPhosphorus Compounds	Carbamates
Methoxychlor DDT HCH (BHC) Lindane Chlordene Hepatochlor Dieldrin Aldrin	Chlorthion Diazinon Dioxathion Dimethoate EPN Malathion (OMS-1) Fenthion (OMS – 2) Methylparathion Parathion Ronnel Trichlorfos Dichlorvos, Chlorpyrifos	Carbaryl Pyrolan Dimetilan Propoxur Synthetic Pyrethroids

Organophosphorus compounds

1. Abate- O,O,O'O' tetramethyl O,O thiodi-p-phenylene phosphorothioate
2. Azinpho-methyl - S (2,4-dihydro 4 oxobenzo (d) (1,2,3,) trizin-3 yl methyl) O,O dimethyl Phosphorodithiote
3. Bidrin – 3- hydroxyl-N,N-dimethyl cis-crotonamide dimethyl phosphate.
4. Carbophenothion – S- (p-chlorophenyl thiomethyli) O,O diethyl-phosphorodithionate
5. Chorothion- O,O dimethyl O (3-chloro 4 nitrophenyl) phosphorothioate
6. Coumaphos – O,O diethyl O-{3-chloro 4 methyl 2-oxo 2H -1 – benzopyran 7 –yl} phosphorothioate
7. DEF- S,S,S – tributyl phosphorothioate
8. Demeton – beta ethyl mercapto ethyl diethyl thiono phosphate
9. DFP – Di isopropyl p[hosphorofluoridate
10. Diazinon – O,O diethyl O-(-2- isopropyl 4 methyl-6-pyrimidyl) phosphorothioate
11. Dicapthon- O,O dimethyl O-(2- chloro -4- nitrophenyl) phosphorothioate
12. Dichlovos- O 2,2 dichlorovinyl O,O dimethyl phosphate
13. Dimethoate- O,O dimethyl S(N- methyl carbamoyl methyl) phosphorodithioate
14. Dioxathon- S,S p-dioxane-2,3 diyl-O.O.-diethyl phosphorodithioate
15. Disulfoton – O,O diethyl S(2-erhylthiol-ethyl) phosphorodithioate
16. Echothiophate- 2 diethoxy phosphinyl thioethyl trimethyl ammonium
17. EPN – Ethoxy-4-nitro phenoxy phenyl phosphine sulphide

18. Ethion- O.O.O’O’-tetraethyl S,S’-methylene bis phophorodithioate
19. Fenthion – O,O dimethyl O(4-(methyl thio)-m-tolyl) phosphorothioate
20. Malaoxon- S- (1,2 bis (ethoxy carbonyl)-ethyl) O,O dimethyl phosphorothioate
21. Malathion- S(1,2 bis (ethoxycarbonyl)-ethyl) O,O –dimethyl phosphorodithioate
22. Menazon- S- (4,6, diamino-s- triazin-syl) methyl O,O- dimethyl phosphorodithioate
23. Merphos- tributyl phosphorotrithioate
24. Methyl demeton- O,O- dimethyl S-(2-ethyl sulfinyl)ethyl phosphorothioate

PATHOPHYSIOLOGY AND CLINICAL MANIFESTATIONS OF OP POISONING

Organophosphorus compounds[13,18] are irreversible anticholinesterases and cause signs and symptoms of **cholinergic excess.**

MAIN ACTIONS / EFFECTS:

1. **Muscarinic** or hollow organ parasympathetic manifestations.
2. **Nicotinic** or autonomic ganglionic and somatic (NMJ) effects and
3. Central nervous system (**CNS**) effects

1. MUSCARINIC EFFECTS[18,19]

Bronchial tree: Tightness in chest, rhinitis, dyspnoea, cough, wheezing suggestive of bronchoconstriction, increased bronchial secretions, pulmonary edema, cyanosis.

Gastrointestinal tract: Nausea, vomiting, cramps, diarrhea, tenesmus, faecal incontinence

Sweat gland: Increased sweating

Salivary gland: Increased salivation

Lacrimal gland: Increased lacrimation

Cardiovascular system: Ventricular fibrillation and ventricular tachycardia

Pupils: Miosis

Ciliary body: Blurring of vision

Bladder: Frequency / urinary incontinence

2.NICOTINIC EFFECTS

Striated muscles– Muscular twitching, fasciculation, weakness, cramps and paresis including that of muscles of respiration (fasciculation – acetylcholine action is for short time – 200 μ.sec. : Hence first single wave of depolarisation occurs, then rest of Ach is taken up by vesicles or destroyed by cholinesterases. But because of persistent

stimulation this synchrony is lost, leading to asynchronous excitation, which causes fibrillation of muscle fibres later leading to paresis.[18,19]

Sympathetic ganglia – pallor, tachycardia, increased BP.

3.CENTRAL NERVOUS SYSTEM

Giddiness, tension, anxiety, restlessness, emotional lability, excessive dreaming, insomnia, headache, tremor, depression, drowsiness, confusion, slurred speech, generalized weakness, coma with absence of reflexes , Type I paralysis, Cheyne-Stokes respiration, convulsions, depression of respiratory and circulatory centres with dyspnoea, cyanosis and fall in Blood pressure.[18,19]

CLINICAL EFFECTS OF OP COMPOUNDS :

PORTAL OF ENTRY: Oral, skin, Inhalation.

ODOUR:

Garlic like odour but the organic solvents which are used to mix organophosphorus compounds may mask it and in fact may smell of kerosene.[15]

EYE:

Miosis is a characteristic sign found in many patients with severe and moderately severe poisoning, but it is not invariably present. Mydriasis can also be seen. Fear, anger, pain and some other emotional stimuli are known to cause dilation of the pupil due to excessive stimulation of the sympathetic nervous system. Regardless of the mechanism, the size of pupil is subject to numerous influences and it would appear unwise to regard pupillary status alone as essential for diagnosis or as important for measuring the success of treatment.[20]

CARDIO VASCULAR SYSTEM:

The cardiac toxicity associated with organophosphate poisoning is caused by more than one mechanism. Possible mechanisms include sympathetic and parasympathetic overactivity, hypoxemia, acidosis, electrolyte derangements, and a direct toxic effect of the compounds on the myocardium[21].

Tachycardia and increased blood pressure occur in initial stage and bradycardia with low blood pressure in the late stage. Commonest effect observed was tachycardia but late onset bradycardia is attributed to direct action on myocardium by organophosphorus compound. Hypertension occurs due to the combined effect of vasoconstriction from cholinergic stimulation of sympathetic ganglia and noradrenaline release from adrenal medulla. Hypotension may also occur due to muscarinic action or blocking of ganglia by hyperpolarization. Cardiac manifestations including atrial fibrillation, conduction block and ventricular fibrillation and flutter usually occur in the terminal stage.

The cardiovascular actions of anticholinesterases are extremely complex, as they reflect at any moment the algebraic sum of the excitatory and inhibitory actions of the accumulated endogenous acetyl choline at several levels of the innervation of the heart and blood vessels viz., at ganglionic, medullary, vasomotor and cardiac centres. One of the major factors responsible for ECG changes is myocardial injury.

ECG changes seen are sinus bradycardia, right axis deviation, AV block, ST segment depression in all leads and T wave inversion. A combination of metabolic and electrolyte derangements cause myocardial injury. Autonomic dysfunction and asynchronous repolarization produce variable QRS morphology and varying R-R interval. Sinus bradycardia could be due to effect of serum cholinesterase inhibition, acting either directly on the myocardium and conducting tissues or through

neurogenic mechanisms. Right axis deviation could be related to pulmonary edema. ST segment and T wave inversion has been suggested to be due to potassium shift or disturbance of other ions transport across membrane.

RESPIRATORY SYSTEM

Respiratory arrest, a common terminal manifestation of organophosphorus poisoning is produced by overstimulation of receptors at **three levels**.

1. It can be recalled that **Muscarinic** action produces increased bronchial secretions, rhinorrhoea, bronchospasm and laryngospasm, which can result in airway obstruction.[22]
2. On other hand, **Nicotinic** effects are associated with paralysis of the respiratory muscles. Weakness of the muscles of the tongue and pharynx aggravate the upper airway obstruction.[22]
3. Finally there may be **Central depression** of respiration leading to cessation of breathing. This is due to direct action of the organophosphate at the cholinergic synapses in the brain stem that are involved in the control of respiration. *Thomas Chang Yao Tsao et. al.*, observed that the cardiovascular collapse was due to depression of the circulatory centre or due to profound hypoxemia, hypercapnia and acidemia from respiratory failure.[22] *Goswamy R et. al.*, found miosis, unconsciousness, fasciculations and low serum cholinesterase level to be of greatest predictive value for ventilatory support.[11]

GASTRO INTESTINAL SYSTEM

After ingestion of organophosphorus compounds, the initial symptoms may be gastro intestinal, of which increased salivation, nausea, vomiting, abdominal tightness, cramps and diarrhoea are the commonest.

NEURO TOXIC EFFECTS.

After human intoxication by organophosphorus compounds, 4 varieties of neurotoxic effects have been observed:

A. TYPE I PARALYSIS, described by *Wadia et al*, are indicative of acute neurotoxic effects during the cholinergic phase of poisoning. Patients present with giddiness, uneasiness, restlessness, anxiety and tremulousness followed by headache, ataxia, drowsiness, fasciculation, mental confusion and slurred speech.[23]

Fasciculations may be influenced / modified by many factors:

1. The depressed serum cholinesterase levels from the start (to a level just critical for the physiology of neuro muscular junction).
2. Blood flow, acid base balance may influence the effect of excess acetylcholine on the neuro muscular junction.
3. A higher daily dosage of atropine may prevent fasciculations. Signs like impaired consciousness, fasciculations and miosis presenting on admission, will all respond to atropine therapy.

B. TYPE II PARALYSIS, described by *Wadia et al* and referred by *Senanayake* as Intermediate syndrome occurs 24-96 hours (after poisoning with organophosphorus compounds) following the acute stage of cholinergic crisis. The cardinal feature of the syndrome is muscle weakness affecting motor cranial nerves, neck flexors and

proximal limb muscles and is frequently associated with respiratory insufficiency. Deep tendon reflexes are usually absent. Prognosis for recovery is good and occurs within 18 days. However, death is usually due to respiratory paralysis.[23,24]

There are several postulations regarding the mechanism of Type II paralysis:

1. *Wadia et al*. has suggested that persistence of nicotinic effects due to lack of early use of oximes may be responsible for the paralysis.
2. However, in *Senanayake* series all patients had been treated with PAM and atropine. Recently, Wadia et al. has suggested that the nicotinic effects of the organophosphorus compounds for some reason appear later than the muscarinic effects, producing the paralysis. He also observed a low serum cholinesterase level in serum at the time of admission in almost all cases that developed paralysis.[23,24]
3. Godath and Fisher attributed the late onset of paralysis to the release of organophosphorus compounds from the adipose tissue acting on the nicotinic receptors.[25]

C. DELAYED POLY NEUROPATHY, described by *Senanayake*, seems uncommon in India, manifest after a latent period of 2-3 weeks following the acute poisoning. The polyneuropathy is predominantly distal, affecting lower limbs before the upper limbs are affected. Paraesthesias are present and muscle wasting is seen. Electrophysiological studies reveal evidence of denervation. Delayed neurotoxicity is a two step phenomenon. First step is the phosphorylation of the protein neurotoxic esterase (NTE), normally present in nervous tissue. Second step is ‘ageing’ of the phosphorylated enzyme complex. A high level of inhibition (70-80%) of the NTE is possibly necessary for the neurotoxicity.[26]

Comparison of the Intermediate syndrome and delayed neuropathy[19]

	Intermediate Syndrome	Delayed Neuropathy
Latent period	1-4days	2-3 weeks
Sites of Weakness	Proximal	Distal
Limb muscle	+	−
Neck	+	−
Cranial	+	−
Respiratory	+	−
Electromyogram	Tetanic fade	Denervation
Recovery	4-18 days	6-12 month
O.P. agents commonly involved	Fenthion , Dimethoate	Methamidophos, Trichlorphos

D. LANDRY – GUILLIAN BARRE (LGB) SYNDROME: The last variety of neuro toxicity is also seen is the Landry – Guillian Barre (LGB) syndrome . It has all classical features of LGB (Clinical, CSF and EMG findings) minus the presence of any other antecedent event. But the prognosis is good in the syndrome seen after exposure to organophosphorus compounds.[27]

REBOUND PHENOMENON

It is often observed that cases which apparently recover in terms of level of consciousness and pulmonary edema fall back into a terminal phase. This has been attributed to toxic myocarditis and intestinal reabsorption of organophosphorus compounds .Compounds can remain bound to fat or adipose tissue for a long time and their sudden release result in rebound phenomenon. The cause of release is:

1. The continuing absorption of the toxin after the transient effect of oximes has subsided and
2. Early reduction atropine dosage when serum cholinesterase depression is still present.

THE ROLE OF ESTIMATION OF SERUM CHOLINESTERASE :

Estimation of acetyl cholinesterase level in circulation is *theoretically preferred* in organophosphorus poisoning since it would reflect the degree of inhibition of synaptic cholinesterase at motor end plates. But, in practice, estimation of serum cholinesterase has an advantage because the measurement is simpler and more accurate than estimation of the acetylcholinesterase. Serum cholinesterase levels can indicate the prior presence of cholinesterase inhibition even after recovery of acetylcholinesterase activity by Pralidoxime in organophosphorus poisoning.[18]

In acute poisoning, manifestations generally occur only after more than 50% of cholinesterase is inhibited. The severity of manifestation parallels the degree of inhibition of serum cholinesterase (SCE) activity pertinently only in initial stages.[18]

The normal values range between 2180 to 9180 IU / L. According to Proudfoot,[28,51] the organophosphorus poisoning may be classified based on the levels of serum cholinesterase (SCE) on presentation as follows :

- In mild poisoning: SCE level is reduced by less than 10%
- In moderate poisoning: SCE level is reduced by 10% to 50%
- In severe poisoning: SCE level is reduced by more than 50%

In severe poisoning, return of normal levels requires above 4 weeks for serum cholinesterase and about 10 to 12 weeks for acetylcholinesterase.

Acetylcholinesterase regenerates at approximately 1% per day, whereas serum cholinesterase regenerates at a more rapid rate, at approximately 25% in the first 7-10 days.

The confirmation of diagnosis depends on demonstrating reduced cholinesterase activity in the circulating blood. Activity is expressed as percentage of normality of healthy adults. Values above 80% normality imply that no significant absorption has taken place. There is no specific upper limit, cases with values between 50% and 80% are usually symptomless but indicate a slight absorption. Early effects, mainly abdominal discomfort and cold sweats may occur at 20% - 30% of normality. Severe cases would be expected to have very low serum cholinesterase activity in the blood, for example 5-10% but some apparently severe cases of parathion poisoning have had upto 50% normal values, while some cases of over exposure, with serum cholinesterase levels reduced to 5 % normality, have proved symptomless.[19]

CHANGES IN ACETYLCHOLINESTERASE LEVELS DURING POISONING AND TREATMENT:

∗ **Serum cholinesterase inhibition** depends on the concentration of the inhibitor, as this is subject to continuous unknown fluctuations and it is not possible to predict the time course of inhibition. Enzyme inhibition will proceed until a steady state is reached and spontaneous reactivation is achieved.

∗ **Cholinesterase activity of red blood cells** is instantly and completely restored and long lasting, but the return of activity of serum cholinesterase (SCE) is transient and variable after oximes. (The main effect produced by the administration of oximes is the restoration of the true acetylcholinesterase activity & prompt and complete relief of symptoms, especially after alkylphosphate poisoning. True cholinesterase level indicates effectiveness and serum cholinesterase levels indicate the prior presence of cholinesterase inhibitor.[18]

∗ **Time of ingestion & relation to serum cholinesterase activity**: A definite correlation between time of ingestion and serum cholinesterase activity is found viz., longer the interval lower the activity. It appears that in doubtful cases or in cases with bizarre clinical picture, and in cases where more than one poisonous substance is ingested, the estimation of serum cholinesterase activity would be of some importance in the diagnosis of the case. Perhaps individual response to excessive cholinergic activity is very variable at identical levels of serum cholinesterase activity.

DISEASES AS A SOURCE OF VARIATION ON THE SERUM CHOLINESTERASE ACTIVITY:

Normal levels are seen in uncomplicated obstructive jaundice, myasthenia gravis, hyperthyroidism, asthma, hypertension, epilepsy and diabetes mellitus

Low levels are observed in patients with parenchymal liver disease, thiamine malnutrition, etc. In patients with liver disease not only do they have decreased levels but a further decrease ensues as a result of exposure to an organophosphorus compound. Serum cholinesterase is sharply reduced in acute myocardial infarction and below normal in dermatomyositis. Nephrotic syndrome patients have increased levels of serum cholinesterase.

DISADVANTAGES OF SERUM CHOLINESTERASE ESTIMATION:

1. Normal values of serum cholinesterase are widely variable from one person to another as well as in the same individual at different times.
2. Low serum cholinesterase levels have been observed in some disease states and may also be genetically determined.
3. Following pralidoxime administration, true cholinesterase levels indicate the effectiveness of PAM and serum cholinesterase levels indicates prior presence of cholinesterase inhibition even after recovery of true cholinesterase activity by PAM, hence the latter cannot be used to assess the effectiveness of PAM therapy.
4. Serum cholinesterase level at a particular time in the blood is not constant but continuously changing as the inhibition of the enzyme by inhibitors and spontaneous reactivation will take place simultaneously.

MANAGEMENT OF ORGANOPHOSPHATE POISONING

Treatment includes

1. Maintenance of vitals
2. Minimizing further absorption
3. Specific antidotes to counter the effects of poisoning
4. Supportive measures

1. Maintenance of vitals:

Ensure a patent airway by frequent suctioning. Endotracheal intubation may be necessary to protect the airway from aspiration and guarantee the adequate tissue oxygenation. Maintain haemodynamic status using the specific treatment.

2. Minimizing the further absorption:

Dermal exposure[29,30]:

Contaminated clothing should be removed from the body, skin should be washed thoroughly with soap and water or with hypochlorite (household bleach). A skin decontaminating kit applicator pads impregnated with ambergard 555 ion-exchange resin and activated charcoal are rubbed over contaminated skin and discarded.

Gastric lavage:

Gastric lavage should be done and is most effective within 30 minutes of ingestion of poison. Protect airway by placement in Trendelenburg and left lateral decubitus position or by endotracheal intubation.[31]

Lavage fluid:

a) Lavage with 150 to 200 milliliters warm saline per wash (in children or adults) and 10 milliliters/kilogram body weight of normal saline in children. Continue until lavage return is clear.
b) The volume of lavage return should approximate amount of fluid given to avoid fluid-electrolyte imbalance.
c) Caution: avoid the risk of electrolyte imbalance and water intoxication and hypothermia in very young children and the elderly.

Complications:

- Aspiration pneumonia
- Hypoxia
- Hypercapnia
- Mechanical injury to the throat, esophagus, or stomach
- Fluid and electrolyte imbalance.

Activated Charcoal:

Charcoal as slurry (240mL water/30 g charcoal).

Usual dose: 25 to 100 g in adults/adolescents, 25 to 50 g in children.

3. Specific antidotes of organophosphorus poisoning

There are two antidotes available for treatment of organophosphorus poisoning

1. Anticholinergic agents
   - Atropine sulfate
   - Glycopyrrolate
2. Oximes

Atropine:

According to WHO, recommended doses of Atropine are as follows.

Mild cases	:	1-2mg IM/IV repeated every 30 minutes.
Moderate cases	:	2-4mg IM/IV repeated every 10 minutes.
Severe cases	:	4-6mg IM/IV repeated every 3-8 minutes.

Dose should be repeated till signs of atropinization (drying of pulmonary secretions) occur. A state of mild atropinization should always be maintained.[32] Drying of excessive secretions is a preferable indicator for completeness of atropinization rather than heart rate or pupil size, because tachycardia and mydriasis can be signs of nicotinic effects of severe organophosphate poisoning. Also, miosis can persist, thus resolution of miosis should not be used as a therapeutic endpoint. Paediatric treatment with atropine comprises of doses 0.02-0.05 mg/kg every 10-30 minutes. Continuous infusions of atropine can be used in the doses of 0.02-0.08 mg/kg/hr.

If a patient is hypoxic and cyanosed, it should be corrected before atropine administration, in order to avoid the risk of ventricular tachycardia as associated with hypoxia.

Inhalational Atropine:

As an adjunct to intravenous atropine, atropine sulfate 2 milligrams via hand-held nebulizer may be used intermittently to treat local pulmonary effects.

Glycopyrrolate:

A quaternary ammonium compound with its high selectivity for peripheral cholinergic sites, has been a useful drug in organophosphate intoxication for controlling secretions, with minimal side effects compared to atropine. Glycopyrrolate has the advantages of better secretion control, less tachycardia, and inability to cross the blood-brain barrier; but it is less effective in treating bradycardia and may not affect central neurologic effects from organophosphates.[33]

Oximes:

Several oximes have been used in the management of acute orgahophosphorus poisoning. These include

- Pralidoxime
- Obidoxime

Pralidoxime (PAM): -

- is a cholinesterase reactivater
- chemically its 2-pyridine aldoxime methochroride[33]

Types:

- Pralidoxime iodide
- Pralidoxime chloride
- Pralidoxime methyl sulphate

Indications:

Severe poisoning with nicotinic (muscle and diaphragmatic weakness, fasciculations, muscle cramps, etc) and/or central (coma, seizures) manifestations should be treated with PAM in addition to atropine.

Mechanism of action:

1. A direct action converting the inhibitor to harmless compound.
2. A transient reaction protecting the enzyme from prolonged inhibition.
3. Reactivation of the inhibited alkyl phosphorylated enzyme to free the active unit.

Pralidoxime does not reverse the muscarinic manifestations of organophosphorus compounds. It has a short elimination half-life of 1.2 hr when given IV. Effect of pralidoxime is seen within 10-40 min. Pralidoxime is probably more effective when administered in the first 1 to 3 hours.

Pralidoxime chloride is a useful agent in the treatment of organophosphate poisoning. Poisindex, a widely used poisoning treatment resource, recommends dosing pralidoxime chloride as an intermittent IV infusion every 8-12 hours, whereas others have used continuous iv infusion with good results. Available animal data suggest that a serum concentration of 4 micrograms/ml may be a minimal level to protect against the toxic effects of organophosphates. Pharmacokinetic simulations, based on parameters obtained from healthy non-poisoned subjects, show that pralidoxime levels fall rapidly to less than 4 micrograms/m1 within 1.5-2 hours after a 1-g IV bolus. Continuous IV infusion (0.5g/h) maintains pralidoxime levels greater than 4 micrograms/ml throughout the length of infusion.

Dosing:

a) Adult - 1 to 2 grams intravenously mixed in 250 milliliters of normal saline and infused over 20 to 30 minutes, In view of short elimination half-life of pralidoxime, a continuous infusion of 0.5 gm/hr would produce constant therapeutic concentration. In cases where intravenous administration is not

possible, pralidoxime can be given intramuscularly at an initial dose of 1 gram in 3 milliliters of diluent.

b) Child - 20 milligrams per kg diluted to a 5 percent concentration in normal saline and infused.

Dosing Intervals – can be repeated with lgm after 20 min if no improvement is seen.

Maximum. Dose - The maximum recommended dose for pralidoxime is 12 grams in 24 hours for adults (British National Formulary, 1988).

Adverse Reactions:

a) Minimal toxicity - When administered as directed, pralidoxime has minimal toxicity. Dizziness, nausea and dry mouth may occur.
b) Neuromuscular blockade - High doses have been reported to cause neuromuscular blockade.
c) Visual disturbance - Oximes may produce visual disturbances and transient increase in intraocular pressure.
d) Asystole - Pralidoxime administered intravenously at an infusion rate of 2 grams over 10 minutes may result in asystole.
e) Atropine side effects - Concomitant administration of pralidoxime may enhance the side effects of atropine administration.

Obidoxime:

Obidoxime dichloride may be a less toxic and more efficacious alternative to pralidoxime in poisonings from organophosphates containing a dimethoxy or diethoxy moiety. It is apparently favored over pralidoxime in clinical practice in

Belgium, Israel, The Netherlands, Scandinavia, and West Germany and is the only oxime available in Portugal.[33,34]

1. Obidoxime may be given as an intravenous or intramuscular injection of 250 milligrams to achieve a therapeutic blood level of 4 milligrams per liter.
2. Subsequent injections of 250 milligrams every 2 hours or continuous infusion of 35 milligrams per hour.
3. Adverse effects - of obidoxime in humans may include hypertension, facial warmth, and numbness.

4. Supportive measures:

- Supplemental oxygenation,
- Frequent suctioning of secretions,
- Fluid replacement to prevent dehydration.
- Antibiotics to check aspiration pneumonia and nosocomial infections.
- Inhaled beta agonists to treat bronchospasm if atropine alone is inadequate.
- Standard antiarrhythmic agents may be required to treat dysarrhythmias.

COMPLICATIONS OF ORGANOPHOSPHORUS POISONING

MANAGEMENT:

- **Seizures**

Administer a benzodiazepine

Diazepam IV (Adult: 5 to 10 mg, repeat every 10 to 15 min as needed.

Child: 0.2 to 0.5 mg/kg, repeat every 5 min as needed)

If seizures recur, Phenobarbital 30 mg (adults) or 10 mg (children > 5 years).

- **Pulmonary Edema (Noncardiogenic):**

Maintain ventilation and oxygenation and evaluate with frequent arterial blood gas or pulse oximetry monitoring. Early use of PEEP and mechanical ventilation may be needed.

- **Hypotension:**

Infuse 10 to 20 ml,/kg isotonic fluid

Place in trendelenburg position.

If hypotension persists, administer dopamine (2.5 to 20 mcg/kg/min) or norepinephrine (0.1 to 0.2 mcg/kg/min), titrate to desired response.

- **Eye Exposure:**

Irrigate exposed eyes with copious amounts of tepid water for at least 15 minutes.

Drugs contraindicated in organophosphorous poisoning :

- Succinylcholine
- Phenothiazines
- Antihistamines
- Opiates
- Barbiturates
- Epinephrine
- Aminophyline.

REVIEW OF LITERATURE

GoelA et. al., in their study of 103 patients with Organophosphorus poisoning found that 35 % required ventilation and correlated the need for ventilation with various clinical parameters[35].

TsaoTC et al., found over a five year period that respiratory failure developed in about 40 % of patients of Organophosphorus poisoning or carbamate poisoning and half of them died due to respiratory failure[22].

Egorov VM, Medvinskiĭ ID, Novikova OV, Tikhonova SA. analyzed the respiratory complications in patients with acute respiratory failure (ARF) developing as a result of poisoning with organophosphorous (OPC). Twenty-two percent of 470 patients had to be maintained on forced ventilation because of ARF. The incidence of respiratory complications among patients on forced ventilation was as high as 92%, which is due to the pathogenesis of the chemical disease developing after OPC poisoning. The respiratory distress syndrome of adults ranks first among the respiratory complications. This syndrome is one main cause of high mortality, particularly so in subjects dying in three or more days of intensive care[36].

Molly J Coye et.al., followed up three groups of agricultural workers exposed to Organophosphorus pesticides , in the absence of baseline values for cholinesterases (RED CELL and SERUM CHOLINESTERASE). They found that both red cell and serum cholinesterase values were decreased during the follow up period. They also confirmed that in the absence of baseline values, interpretation of single post exposure value is not useful because of the wide range of normal cholinesterase activity and advised sequential estimations to be better indicators[37].

Goswamy R. et al., assessed the clinical profile and serum cholinesterase levels in Organophosphorus and carbamate poisoning patients and the need for ventilatory support. They concluded that apart from clinical indicators, low serum cholinesterase levels were of greatest predictive value for ventilation in Organophosphorus poisonings[11].

Proudfoot.A, classified the severity of organophosphorus poisoning based on serum cholinesterase levels[28].

Lee P, Tai D.Y., studied clinical features and evaluated the APACHE II scoring system as an alternative index for measuring severity of Organophosphorus poisoning along with estimation of serum cholinesterase levels as a predictor for weaning from mechanical ventilation. Threshold values for weaning were found to be 2900 IU / L and 7500 IU / L[38].

Senanayake N, De Silva H J, Karalliedde L. A scale to assess the severity of organophosphorus intoxication: POP scale. Hum Exp Toxicol 1993; [13]; 297-299[39]

Aygun D et al., found that serum cholinesterase level estimations are useful in diagnosis of Organophosphorus poisonings in acute phase but shows no relation to severity of poisoning[12].

Semir Nouira et . al., estimated serum cholinesterase levels at the time of admission after acute Organophosphorus poisoning with the aim to determine whether this has got a prognostic value with reference to severity, treatment, APACHE scoring and need for ventilation. They found no correlation between serum cholinesterase levels and Organophosphorus poisoning as per the above assessments[40].

A.Ramani et.al., studied 25 cases of Organophosphorus poisoning and found a good prognostic value for serum cholinesterase from samples drawn at regular intervals[41].

Eddleston M (2008) et al, assessed usefulness of butryl cholinesterase activity on admission for predicting severity in acute OP poisoning and found that plasma butryl cholinesterase activity on admission can provide useful information and can be used to predict death when insecticide ingested is known[42].

Reihman S et al. in 2008 conducted a study at Bir hospital , Nepal from Aug 2004 to Sep 2005 and studied 50 patients. Patients were grouped into mild, moderate, severe poisoning according Peradeniya OP poisoning (POP) scale. There were 26% patients in moderate poisoning and only 4% pts in severe poisoning. 14% of the total patients died. The severity of poisoning directly correlated with serum cholinesterase levels ($p<0.001$). The POP scale and serum cholinesterase at presentation appeared useful to assess severity of poisoning, particularly in terms of higher amount of atropine, prolonged duration of hospital stay and need for mechanical ventilation[43]**.**

Ha YR et al., concluded that as an early prognostic factor for the length of ventilator support in organophosphorus poisoning, 1) level of consciousness and 2) serum cholinesterase level at admission, 3) recovery to more than 500 IU/ml within initial 3 days are useful. Especially when the serum cholinesterase level is not recovered to more than 500 IU/ml within initial 3 days, it is essential to observe closely for the possibility of an intermediate syndrome[44].

G. Avasthi, G. Singh, evaluated that at admission, level of serum cholinesterase of less than 200 units is a predictor and the 30 Hz repetitive nerve stimulation (RNS) decrimental response could be a useful marker for the 'Intermediate Syndrome[45].

Shivkumar S, Raghavan, concluded that serum cholinesterase level was valuable in assessing the prognosis as mortality was higher in patients with severe

poisoning with serum cholinesterase level less than 10% when compared to those with more than 10% (65% Vs. 31.5%)[46].

M.S.Manu et al; concluded that the serial measurements of serum acetylcholinesterase levels can be useful in predicting the length of ICU stay, duration of mechanical ventilation and the prognosis of the patient with organophosphorus poisoning[47].

Basar Cander et al; concluded that although serum cholinesterase values can be used in the quick diagnosis, their efficacy at predicting outcome in patients with organophosphorus poisoning has not been established. It has also been determined that serum leukocyte values have no prognostic value in organophosphorus poisoning, but GCS values have been found to be effective in predicting the outcome[48].

Chen H Y et al; proposed that the absence of elevating serum cholinesterase activity level within 48 hours of poisoning appears to associate with higher mortality in acute organophosphorus poisoned patients[49].

Yarden t et al; found that initial SChE level is related to the clinical severity but not with mortality. However S100B may be a useful marker in assessment of clinical severity and prediction of mortality in acute OP poisoning[50].

MATERIALS AND METHODS

This is a prospective study conducted at the intensive care unit of department of anaesthesiology, Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapur, from October 2013 to September 2015. 70 patients presenting with history of of Organo-phosphorus poisoning and features of respiratory failure requiring ventilatory support were included in the present study. Prior approval for the study and the protocol was obtained from the institution ethical committee. After explaining the possible prognosis in the course of organo-phosphorus poisoning , consent from a responsible attendant/informant of the patient was obtained before the actual study was initiated.

Inclusion criteria

1. Provisional diagnosis of organophosphorus poisoning in a patient irrespective of age / sex , based on history by attenders.
2. Presence of characteristic clinical signs and symptoms of severe grade of OP compound poisoning with clinical evidence of respiratory insufficiency.

Exclusion criteria

1. Poisoning with other compounds along with OP like kerosene, sedatives like opioids, diazepam, barbiturates etc.
2. Organo-phosphorus compound poisoning with H/O alcohol consumption and drug abuse.
3. H/O any chronic liver disease or pancreatic disease.
4. Organophos-phorus poisoning in pregnant females.

5. Patients with history of respiratory disease like bronchial asthma, cardiac disease, neuromuscular diseases like myasthenia gravis or muscular dystrophy or other concomitant illnesses like DM.

Each of the patients with Organophosphorus poisoning were assessed clinically with detailed history and thorough physical examination.

Features correlating to:

a) Severity of organophosphorus poisoning and
b) Respiratory failure (to identify those requiring mechanical ventilation) were assessed.

a) Criteria for diagnosis of severe grade of organophosphorus poisoning: [35]

- Unconsciousness
- Marked miosis
- Loss of pupillary reflex to light
- Muscular fasciculation
- Flaccid paralysis
- Excessive secretions from mouth and nose
- Crepitations
- Respiratory distress

b) Criteria for diagnosis of respiratory failure :

The patients who have features suggestive of severe poisoning as stated above are then assessed for ventilatory support based on following:

- Apnoea
- Obvious Hypoventilation
- Persistent Cyanosis inspite of O_2 supplementation
- Persistent Tachypnoea - Respiratory rate (per minute) > 24

- Persistent SpO_2 < 90% with Oxygen supplementation by non invasive means.
- Active involvement of accessory muscles of respiration

Immediately after clinical assessment, blood samples were sent for investigations including Haemoglobin level , Total count and Differential Blood Count (DC), ESR, Blood sugar, blood urea, serum creatinine, serum electrolytes and Serum Cholinesterase level (More investigations as necessary were done after institution of treatment and ventilation).

IMMEDIATE MANAGEMENT:

Patients were given stomach wash, body wash and intravenous cannulation done. Injection PAM, bolus dose – 1 gm. I.V. and Inj. Atropine bolus 2-4 mg. IV were given. Patients were reassessed for respiratory failure, and if so, intubated and shifted to Intensive Care unit by Ambu ventilation.

On arrival in the Intensive Care Unit, the patients were immediately connected to ventilator and *supportive therapy* was also initiated along with *definitive therapy.*

THE DEFINITIVE THERAPY for Organophosphorus poisoning :

Protocol followed was:

- Inj Atropine Infusion 25-50 mg. / 24 hrs, with l mg bolus IV every 2^{nd} hrly as and when required, till signs of atropinization appeared.
- Inj PAM infusion at 50 - 100 mg / hr administered for the initial 72 hrs. depending upon the severity.(following the initial bolus 1 gm. given on arrival at emergency ward)

SUPPORTIVE THERAPY (as required):

- Maintenance of intravascular volume by IV fluids.
- Antibiotics to prevent and treat infections.
- Inotropic support for cardiac function.

- Regular and thorough endotracheal and oral suction.
- Chest physiotherapy.
- Nutritional support by enteral feeding when indicated.
- Measures to reduce gastric acidity and secretions.

VENTILATOR MANAGEMENT:

The patients were put on *Teema* ventilator with the following *initial* settings-

Mode - SIMV

Tidal volume - 7-10 ml/kg body wt

Respiratory rate - 10-12 bpm

FiO_2 - 0.4 – 0.6

I : E ratio - 1 : 2

Pressure support - 10-15 cm H_2O initially and gradually reduced to 0 with recovery .

PEEP - 2 to 5 cm H_2O increments was used as indicated

Weaning Technique – Consisted of SIMV with PS ⇒ PSV ⇒ CPAP

Investigations : Repeat and / or additional investigations as required by the patient's status were ordered and Serum Cholinesterase estimation was repeated on the 3^{rd}, 5^{th}, 7^{th}, 9^{th} and 11^{th} day of stay in the Intensive Care Unit.

Data Collection and Analysis:

The patients were assessed based on:

- Demographic parameters and type / time of poisoning
- Symptoms and signs of poisoning
- Severity of intoxication and clinical features of respiratory failure
- Pharmacological therapy
- Ventilation : Modes and other settings

- Monitoring : Clinical and Laboratory

Laboratory: (a) Routine (b) Serum Cholinesterase

The collected data were analysed with special reference to the

1. Severity of poisoning (as assessed in the emergency ward)
2. The treatment
3. The duration and nature of ventilatory support
4. The levels of serum cholinesterase estimated on first day and serially on subsequent odd days .

Correlations among these groups of data are evolved and discussed.

SERUM CHOLINESTERASE:

The reference values and Interpretations / definitions are as follows:

The serum cholinesterase activity was estimated by, ROCHE COBAS C 311 FULLY AUTO ANALYSER, HITACHI.

Reagent used were –

a. Pyrophosphate 95mmol/l, Hexacyanoferrate(III) 2.5 mmol/l, pH 7.6
b. Butyrylthiocholine

EDTA samples are sent to the laboratory.

The results are expressed in IU / L. The laboratory reference range used in the present study for serum cholinesterase **:** 2180 to 9180 IU / Ltr

Based on the Serum Cholinesterase values, the severity of poisoning may be defined as per Proudfoot classification[28, 51] with above normal range:

	Mild	**Moderate**	**severe**
Plasma cholinesterase level	**<10% reduction**	**10% - 50% reduction**	**>50% reduction**

In keeping with this definition, 1962-2180 IU/L can be considered as mild, 1090- 1962 IU/L as moderate and less than 1090 IU/L as severe toxicity.

GRADING OF MOTOR POWER (Medical Research Council Scale)[52]

0 No muscle contraction visible

1 Flicker of contraction but no movement

2 Joint movement when effect of gravity eliminated

3 Movement against gravity but not against examiner's resistance

4 Movement against resistance but weaker than normal

5 Normal power

Outcome

Outcome is assessed by percentage of mortality in OP poisoning patients on ventilator support.

STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistical Package for the social sciences (SPSS) version 16. Results were analyzed using

- Diagrams
- Descriptive statistics (to measure mean, standard deviation)
- Correlation
- Chi square test
- t test

RESULTS

Demographic data

Table (1A)

AGE DISTRIBUTION OF PATIENTS

Age (Yrs)	N	Percentage
15-25	35	50.0
26-40	16	22.9
41-55	11	15.7
>55	8	11.4
Total	70	100

TABLE (1B)

Min Max and Mean of age of patients.

	Minimum	Maximum	Mean	SD
Age(Yrs)	15	79	32.1	15.5

GRAPH (1)

AGE DISTRIBUTION OF PATIENTS

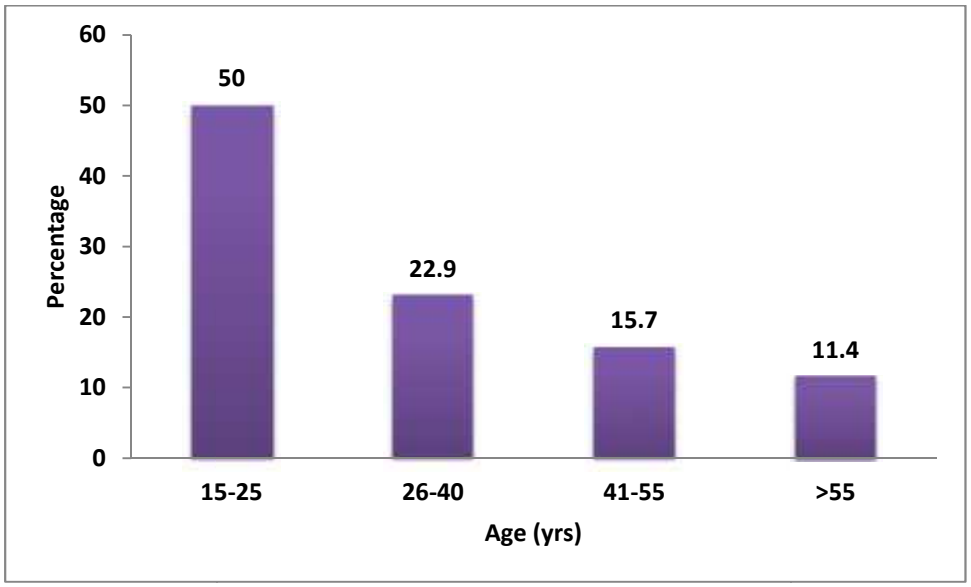


Table 1(a) and (b) shows the age distribution of the patients. Majority of the patients of poisoning in our study (72.9%) were below 40 years of age. The youngest patient was 15 years old and the oldest, 79 years old.(Graph 1)

TABLE (2)

GENDER DISTRIBUTION OF PATIENTS

Gender	N	Percentage
Male	42	60
Female	28	40
Total	70	100

GRAPH(2)

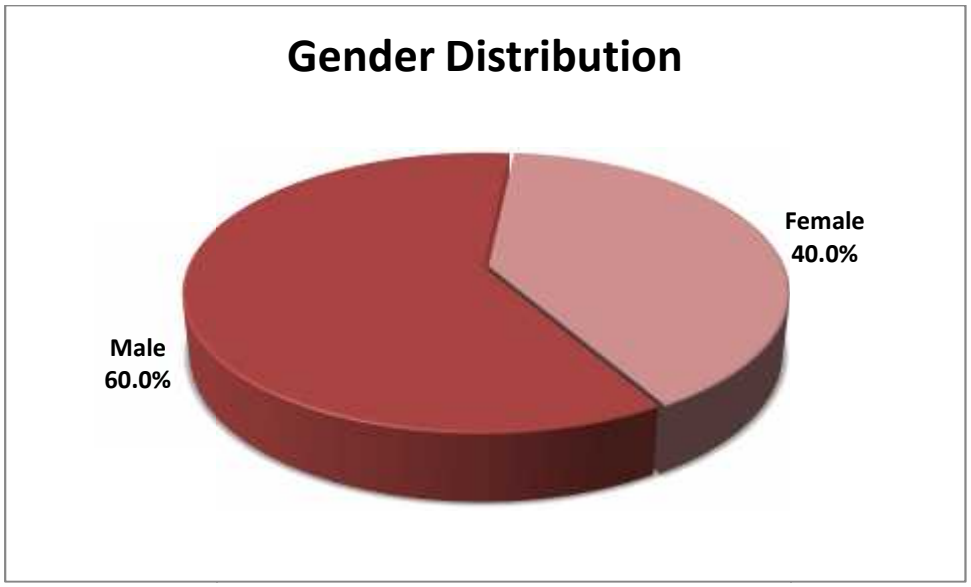


Table 2 shows the gender distribution of the patients. Out of 70 patients included in the present study, 42 (60%) were males and only 28 (40%) were females. (Graph 2)

Table (3)

Nature of poisoning

Nature of poisoning	No. of patients	
	N	**Percentage**
Suicidal	61	87.1
Accidental	9	12.9
Total	70	100.0

Graph (3)

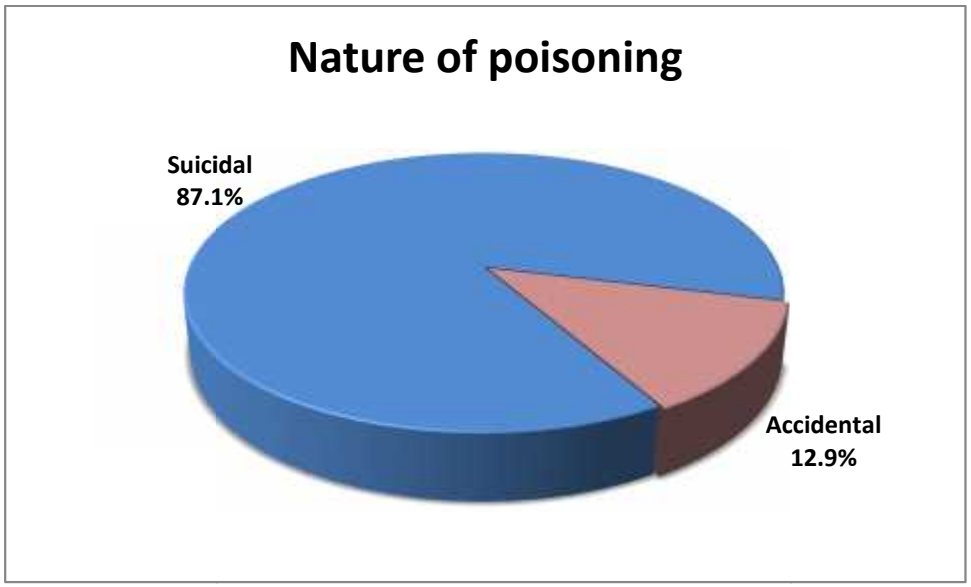


Table (3) and graph (3) shows that out of 70 cases of Organophosphorus poisoning in the present study, majority i.e., 61 out of 70 cases (**87.1%**) were **suicidal** and 9 (**12.9%**) cases were **accidental**.

Table (4)

TYPE OF ORGANOPHOSPHORUS POISON CONSUMED

Type of compound	No. of patients	
	N	Percentage
Monocrotophos	50	71.4
Diclorvas	5	7.1
Endosulfan	2	2.9
Metacid	2	2.9
Phorate	1	1.4
Quinalphos	2	2.9
Ethion	2	2.9
Thimet	1	1.4
Triazophos	1	1.4
Dimethoate	1	1.4
Unknown	3	4.3
Total	70	100.0

50 out of 70 patients took Monocrotophos the rest had poisonings from other types of Organophosphorus compounds.

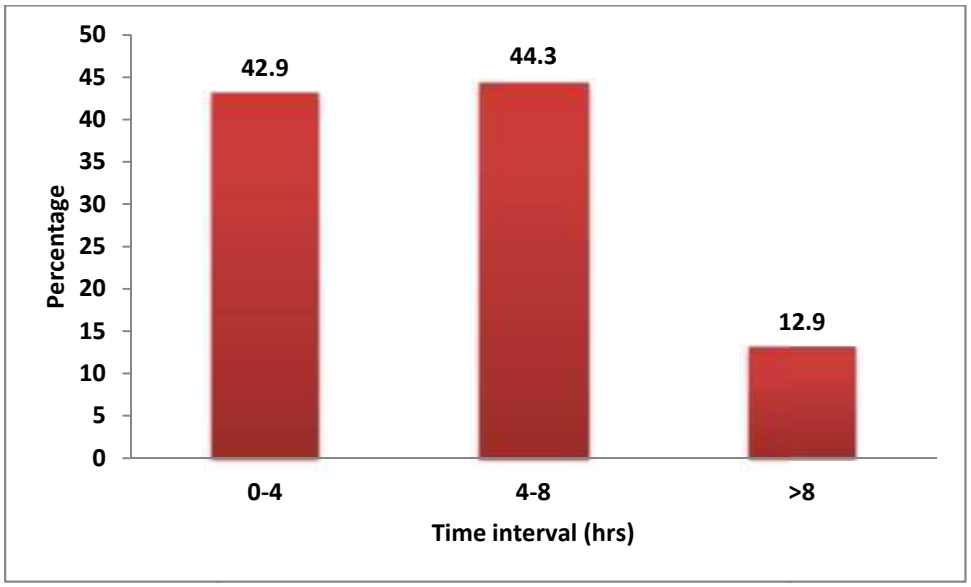
Table (5)

Time Interval from Consumption to Time of Admission to Emergency Ward

TIME INTERVAL (Hrs)	No. of patients	
	N	Percentage
0-4	30	42.9
4-8	31	44.3
>8	9	12.9
Total	70	100

Graph (4)

Time Interval from Consumption to Time of Admission to Emergency Ward



(Table 5 and graph 4) Nearly 57.2 % were brought after 4 hours of poisoning. This could be related to the fact that majority of the patients were from rural areas and agricultural workers by occupation and needed to be brought to the tertiary hospital for management.

Table (6a)

SEVERITY OF O.P. POISONING ON PRESENTATION (DAY 1)

SCE Level* (IU/L)	Severity	N	Percentage
<1090	Severe	45	64.3
1090-1962	Moderate	10	14.3
>1962	Mild	15	21.4
Total		70	100

* (The reference lab. value for serum cholinesterase in the study: 2180 to 9180 IU / L)

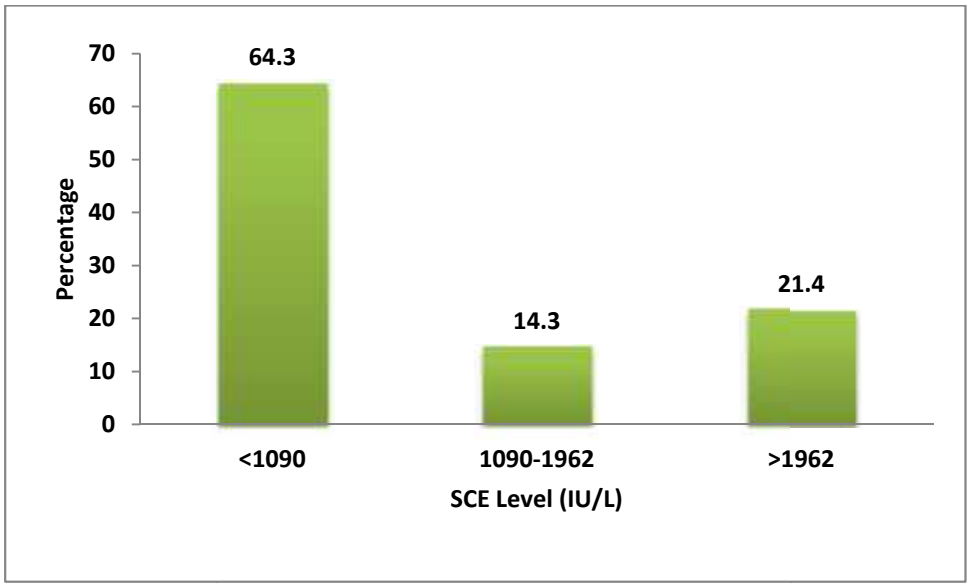
Table (6b)

Min Max and Mean of O.P. POISONING ON PRESENTATION (DAY 1)

SCE Level (IU/L)	Minimum	Maximum	Mean	SD
	129	8901	1445.1	1888.0

Graph (5)

SEVERITY OF O.P. POISONING ON PRESENTATION (DAY 1)



There is reduction / suppression of SCE in all of the patients on the day of presentation (1445.1 $\pm$ 1888.0 IU / L).

It is evident from the above table (6a), (6b) and graph (5) that maximum number of patients presented with severe poisoning based on the SCE levels. (45 out of 70 patients i.e 64 %). About 36 % had moderate to mild poisoning as per their SCE levels.

Table (7)

Relationship between Levels of Serum Cholinesterase at Presentation and Time of Consumption of Poison (hrs)

SCE Level (IU/L)	0-4		4-8		>8		P value
	N	%	N	%	N	%	
<1090	16	53.3	23	74.2	6	66.7	0.279
1090-1962	6	20.0	4	12.9	0	0.0	
>1962	8	26.7	4	12.9	3	33.3	
Total	30	100.0	31	100.0	9	100.0	

Graph(6)

Relationship between Levels of Serum Cholinesterase at Presentation and Time of Consumption of Poison (hrs)

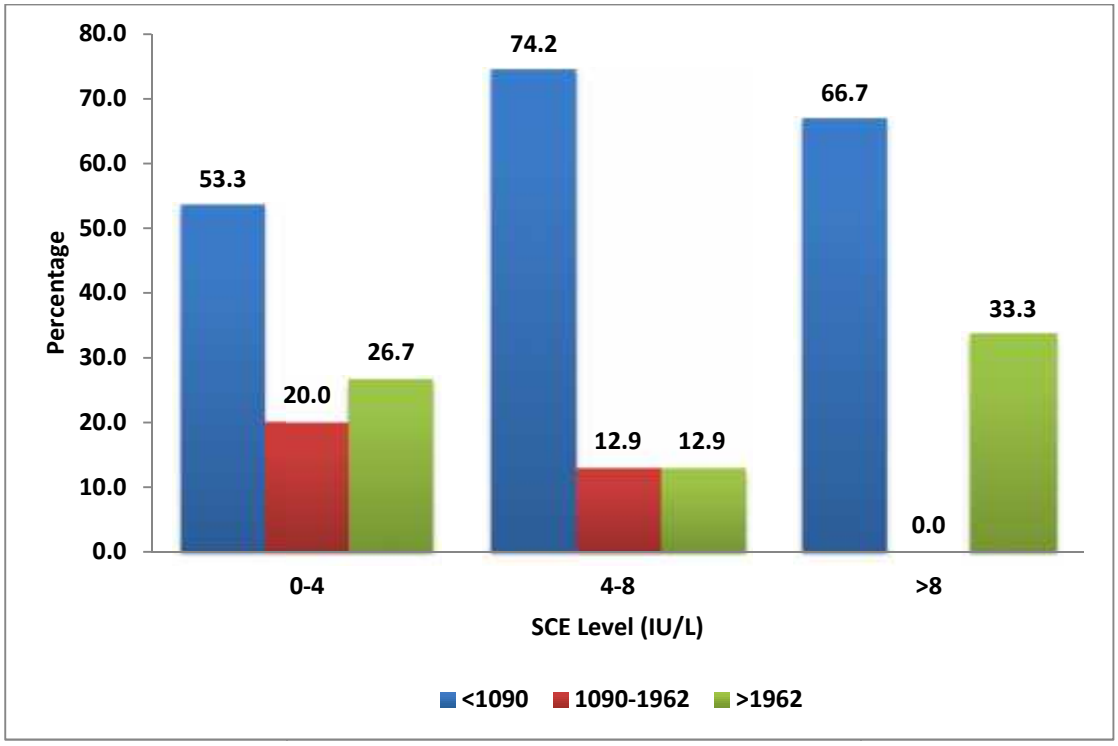


Table (7) and graph (6) shows 61 / 70 of patients (87 %) were brought to the hospital *within 8 hours* of consumption of poison. Based on their SCE values, this group had mild to severe suppression of SCE levels. Significantly, severe suppression of SCE (values < 1090 IU / L) was found in 6/9 patients who were brought after a gap of 8 hours after poisoning.

Table (8)

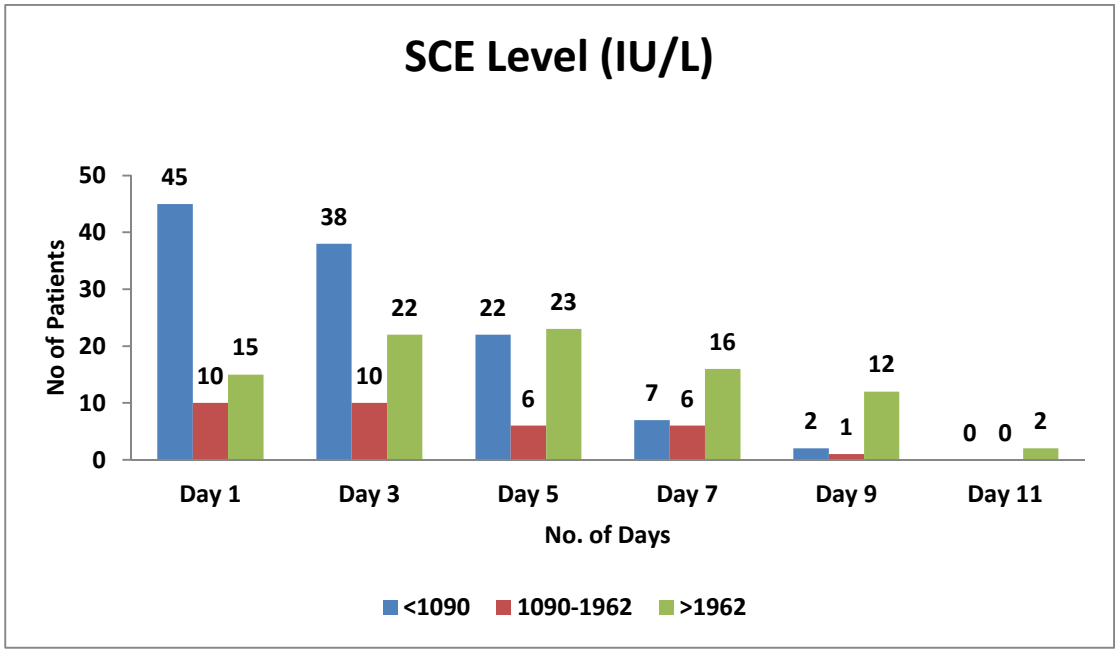
SERIAL SERUM CHOLINESTERASE LEVELS (SCE)*

SCE Level (IU/L)	Day 1		Day 3		Day 5		Day 7		Day 9		Day 11	
	N	%	N	%	N	%	N	%	N	%	N	%
<1090	45	64.3	38	54.3	22	43.1	7	24.1	2	13.3	0	0
1090-1962	10	14.3	10	14.3	6	11.8	6	20.7	1	6.7	0	0
>1962	15	21.4	22	31.4	23	45.1	16	55.2	12	80	2	100
Total	70	100	70	100	51	100	29	100	15	100	2	100

*(The reference lab.value for serum cholinesterase in the study: 2180 to 9180 IU / L,)

Graph (7)

SERIAL SERUM CHOLINESTERASE LEVELS (SCE)



(Table 8, Graph 7) It can be found that there is increase in SCE levels from the first day to the 3rd day and largely stabilizing over the next six days in the set of patients who presented with values of >1962 IU/L. However in the group with 1090-1962 IU/L of SCE levels, there was no improvement in the levels upto 3 days after presentation, indicating that the suppression in those presenting with low values of SCE on presentation will continue to be so for a long time..

Table (9)

Average Change in SCE from first day to subsequent days

	Change in Mean SCE Level (IU/L)	% change
Day1-3	457.3	31.6
Day3-5	261.9	13.8
Day5-7	193.7	8.9
Day7-9	425.9	18.1
Day9-11	-31.4	-1.1

Graph (8)

Average Change in SCE from first day to subsequent days

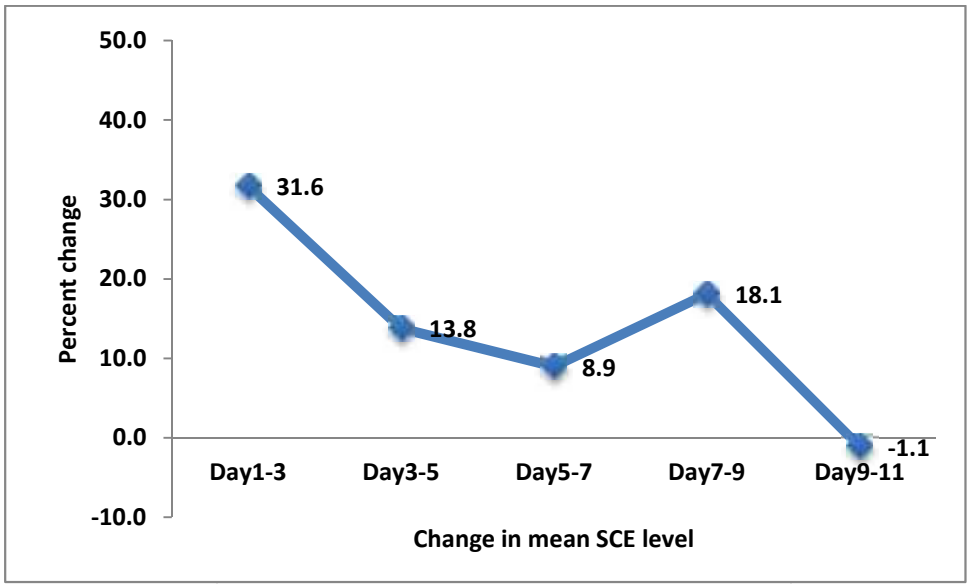


Table 9 and Graph 8 shows the average change in the SCE levels from the time of presentation to the subsequent odd days (till 11th day) as applicable.

We can find that there is increase in the SCE levels from the first day to subsequent days, the maximum increase being seen on third day. These results are illustrated in Graph (8).

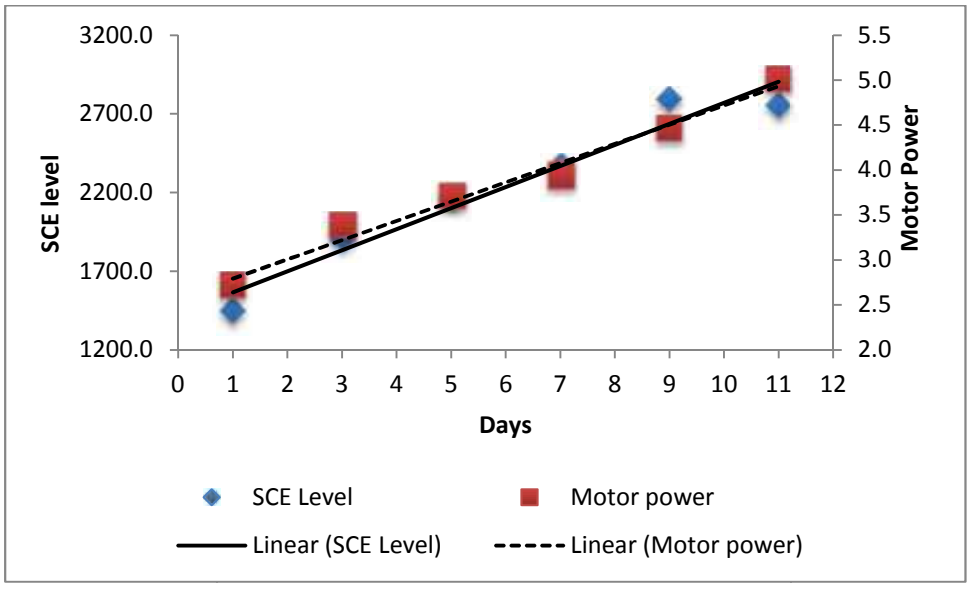
Table(10)

Relation between mean SCE & M. Power

Days	1	3	5	7	9	11
SCE Level (IU/L)	1445.1	1902.4	2164.3	2358.0	2783.9	2752.5
Motor power	2.7	3.4	3.7	3.9	4.	5.0

Graph (9)

Relation between mean SCE & M. Power



There is increase in Serum Cholinesterase level from the first day to the time weaning is started and then to the day of complete weaning.

This increase coincides with a similar increase in motor power being seen over these days (Table 10 and Graph 9)

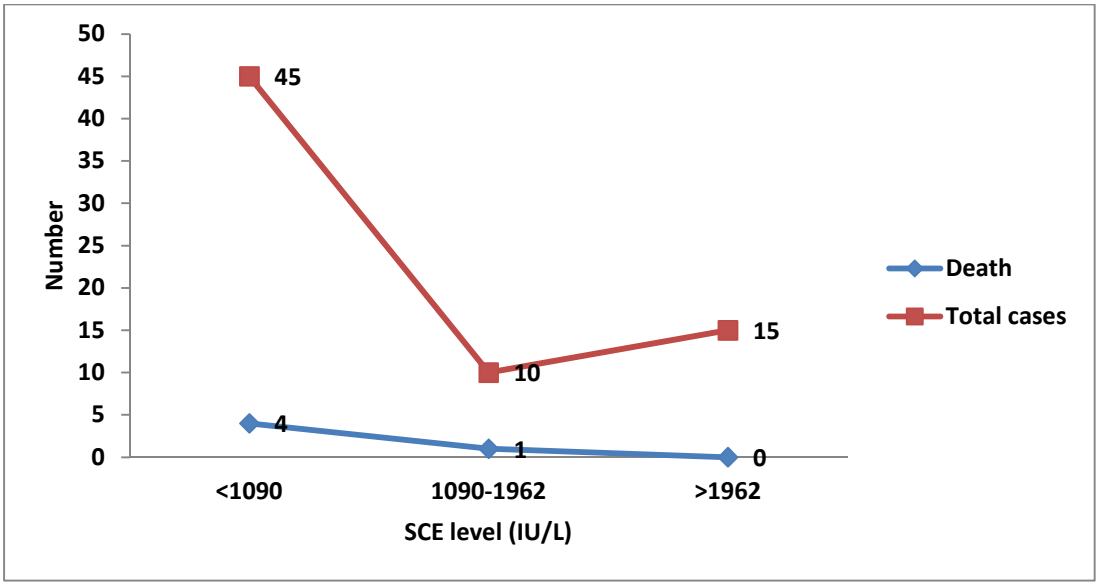
Table (11)

RELATION BETWEEN INITIAL LEVEL OF SERUM CHOLINESTERASE AND MORTALITY

SCE (IU/L)	Total cases	Death
<1090	45	4
1090-1962	10	1
>1962	15	0
Total	70	5

Graph (10)

RELATION BETWEEN INITIAL LEVEL OF SERUM CHOLINESTERASE AND MORTALITY



*(The reference lab.value for serum cholinesterase in the study : 2180 to 9180 IU / L)

7 % of the patients in our study died.

(Table 11)Higher mortality (80%) was seen in pts having severe suppression of SCE activity (Graph 10)

None of the patients died who had mild suppression of SCE levels(0%) (>1962 IU/L).

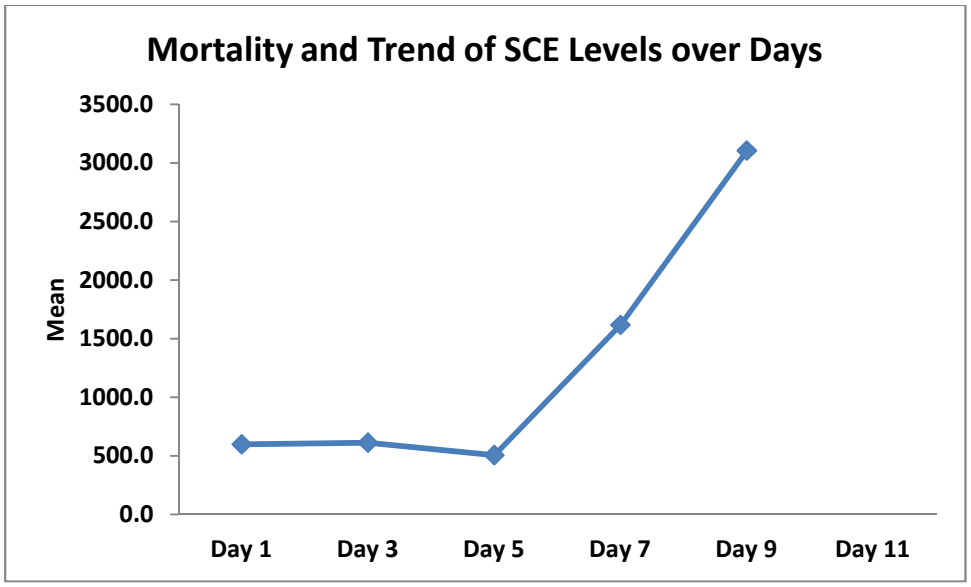
Table (12)

Mortality and Trend of SCE Levels (IU/L) over Days

Days	N	Mean	SD	P value
Day 1	5	599.4	591.0	-
Day 3	5	613.2	560.2	0.971
Day 5	3	507.0	354.8	0.781
Day 7	2	1618.0	1219.1	0.208
Day 9	1	3105.0	-	-
Day 11	0	-	-	-

Graph (11)

Mortality and trend of SCE levels over days



($p < 0.0001$ significant , S : Significant, N.S.: Not Significant)

Table (12)gives the changing values of SCE in the group of patients who died , from the day of admission till death .

There is a fall by fifth day with improvement over the subsequent days (Graph 11). These changes are not however statistically significant.

DISCUSSION

The present study of serum cholinesterase levels in organophosphorus poisoning patients on ventilatory support was conducted in 70 patients at the Intensive Care Unit of the Department of Anaesthesiology at Shri B M Patil Medical College, Hospital and Research Centre, Vijayapur.

In our study majority of the patients were (71%) below 40 years of age and were predominantly males (60%).This could be because males have easy accessibility to organophosphorus compounds. The route of poisoning was by ingestion, with suicidal intent. Majority of the patients were from rural areas and agricultural workers and hence, again easy availability could be the reason for OP poison ingestion. This is consistent with studies of *Shankar P.S*[53] and *Gupta OP et al*[54], who reported the organophosphorus poisoning to be more in males, and maximum incidence of poisoning between age 11 to 30.

Monocrotophos was the commonest type of OP poison used, by more than 72 % of the patients, because of its maximum usage in paddy cultivation in this part of state. Our findings are consistent with studies like *Shankar P.S*[53] and *Gupta OP et al*[54] this part of state.

Nearly 57 % were brought after 3 hours of poisoning. This could be related to the fact that majority of the patients were from rural areas and agricultural workers by occupation and needed to be brought to the hospital for management by road.

SERUM CHOLINESTERASE LEVELS ON THE DAY OF PRESENTATION

In our study, taking into consideration the lower limit of reference values of SCE , only 13 out of 70 patients had SCE values of > 2180 IU / L (18 % of patients), indicating that there was suppression in more than 81 % of patients on presentation (With a reference range of 2180 to 9180 IU / L of SCE).

64 % of patients showed more than 50 % reduction. The mean SCE value was 1445.1 ± 1888.0 IU/L

In a study of 23 patients of OP poisoning *Mehta A.B. et al.*[57] observed lowered activity of SCE in more than 70 % of cases at presentation.

Suvit Areekul et. A[55]studied 10 patients with OP poisoning and found reduced levels of SCE in all of them with one mortality.

A. Dua et al.[56] also found that SCE was lower than normal in all their study patients with OP poisoning .

Thus, reduced levels of SCE on the day of presentation can raise a strong suspicion of OP poisoning .

THE SERUM CHOLINESTERASE AND THE VENTILATORY FAILURE

We have studied only those patients of OP poisoning who had ventilatory insufficiency (and thus considered to have severe poisoning clinically) and were put on ventilatory support .

Majority (> 78.5 %) had moderate to severe poisoning based on Serum Cholinesterase levels (SCE) values, as per Proudfoot classification.[28,51] Only 21.4 % had mild poisoning as per their SCE values .

These findings are in accordance with *Thomas chang yao et al*[22], *Choudhary SC et al*[59], *Kar N*[58] and *Prasad DRMM.*[51]

Tsao TC et al.[22] in their study could associate severe suppression of SCE (> 80 %) with acute / subacute respiratory failure. (<300 mU / ml in a normal range of 3000 to 6000 mU / ml)

Choudhary SC et al.[59] in their study showed that SCE levels were reduced in all the cases and the total dose of atropine required, need for oxygen inhalation, need

for intubation and ventilatory support, mean duration of hospital stay and mortality rate ($P = 0.003$) were higher in moderate to severe cases.

Prasad DRMM et al.[51] in their study also found that cyanosis, muscle weakness, convulsion, respiratory distress and fasciculation were linked to very low SCE levels.

However, *Aygun et al*.[12] found that serum cholinesterase level estimations are useful in diagnosis of OP poisoning in acute phase, but show no relation to severity of poisoning

Semir Nouira et.al.[40] did not find any statistically significant differences in mean SCE levels in those mechanically ventilated and those not needing ventilatory support. They also found that the total dose of atropine required and Simplified Acute Physiology Score (SAPS) used to grade clinical severity in their study did not correlate with the SCE values.

Mehta et a.l[57] also found no correlation between severity of poisoning clinically and SCE levels in their study.

The present study showed that there is correlation between the SCE levels and the clinical severity of poisoning such as presence of ventilatory insufficiency. Also SCE level appears to have prognostic value in patients of OP poisoning .

THE TIME DELAY FROM POISONING TO ADMISSION:

In our study the time interval between consumption of poison and hospital admission ranged from less than 1 hour to more than 12 hours. 42 % of patients were brought within 4 hours of poisoning with mild to severe suppression of SCE levels. The rest (around 58%) brought after 4 hours also had mild to severe suppression of SCE levels..

Our findings are consistent with *Karnik VM*[60] and *Sunder Ram J*[32]. They observed no correlation between severity and time interval.

However *Mehta et. al.*[57] and *Gupta et al.*[54] reported reduced serum cholinesterase levels and increased mortality with increasing time interval between hospital admission and consumption of poison.

SERIAL ESTIMATION OF SERUM CHOLINESTERASE LEVELS:

In those with first day values of > 1962 IU / L of SCE, the serial estimation showed an increase in SCE levels on 3rd day and then largely stabilizing over the next six days. In the set of patients who presented with 1090 to 1962 IU / L of SCE , there was not much improvement in the levels upto 3 days after presentation, indicating continuing suppression with lower levels of SCE at presentation .

There has been no such serial estimation over 11 days in other studies even though this has been done in three individual cases by *Suvit Areekul et al.*[55]

TREND OF CHANGE IN SERUM CHOLINESTERASE (SCE) VALUES

We estimated the average change in the SCE levels comparing with first days readings with the subsequent days (till 11th day), as applicable.

We found that there was a maximum improvement in the SCE levels on the 3rd day – about 31 %. We also found that there was increase, albeit at lesser rates, in the subsequent days as well.

MOTOR POWER AND RELATION TO SCE:

There was an increase in motor power along with improvement in respiration and weaning seen over these days.

In the present study, there was increase in Serum Cholinesterase levels from the first day to the time start of weaning and then to the day when patient was completely weaned off, along with improved motor power.

G. Avasthi, G. Singh[45], while prospectively evaluating 29 patients of OP poisoning clinically along with SCE estimation found that clinically detectable respiratory muscle weakness could be found in all patients with severely depressed values but could still occur at any level of SCE.

RELATIONSHIP BETWEEN MORTALITY AND SERUM CHOLINESTERASE:

There was a mortality rate of 7% in our Study.

In the present study, we found that deceased patients had lowest SCE level at presentation. 80% of the deceased patients had values <1090 IU/L i.e with severe suppression of SCE levels and remaining 20% had moderate levels of SCE suppression. This indicates that there is relation between mortality and SCE levels at presentation.

Likewise, *Chen et al.*[49] showed that low SCE activity with non-rising trend within 48 hours of OP poisoning was associated with higher mortality. Moreover, *Eddleston et al.*[42] revealed that SCE activity can predict death based on the formula of OP compound ingested.

However, *Mehta et al.*[57], *J. Sunder Ram et al.*[32] and *A. Dua et al.*[53] found that mortalities does not correlate with SCE levels.

TREND OF SCE LEVELS IN DECEASED PATIENTS

In the present study we found that the levels of SCE in deceased patients did not change significantly over the days from presentation till death, and also there was no fixed trend.

CONCLUSION

The present study on ‘’Serum cholinesterase levels in Organophosphorus poisoning patients on ventilatory support’’ was undertaken in 70 patients.

There is a male preponderance in our study.

Middle age groups between 20-40 years are more commonly encountered in poisoning by organophosphate compounds.

Majority of the patients were from rural areas who were agricultural workers consuming poison with suicidal intent.

There is no age factor associated with severity of clinical manifestations or mortality.

There was decrease in the level of serum cholinesterase on the day of presentation

There was a generalized increase in the serum cholinesterase levels from first day of poisoning to subsequent days but without a fixed trend.

We found a good correlation between improvements in patient’s motor power with improved Serum cholinesterase levels during the time the patients were successfully weaned.

There is a direct correlation between the level of serum cholinesterase on presentation and clinical severity of poisoning such as presence of ventilator insufficiency.

We found that deceased patients had low levels of SCE at presentation.

However, our study did not reveal any relation between

- Serum cholinesterase levels and the time of presentation after poisoning.
- Any change in trend in Serum cholinesterase levels in those who died during the study period.

SUMMARY

A prospective clinical study was undertaken in patients with a history of organophosphate poison consumption and features of respiratory failure on ventilatory support admitted in the Intensive Care Unit of Department of Anaesthesiology of Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapur from October 2013 to September 2015.

The objectives of the study were to estimate Serum Cholinesterase enzyme levels serially in acute organophosphorus poisoning patients and to correlate the Serum cholinesterase levels to the severity of organophosphorus poisoning and outcome. All patients aged between 15-79 years without any comorbid diseases were eligible for the study. The sample size was seventy patients . Blood samples for serum cholinesterase were collected at the time of admission, 3^{rd}, 5^{th}, 7^{th}, 9^{th} and 11^{th} day.

The study revealed all the patients with OP poison ingestion had low levels of serum cholinesterase levels on the day of presentation to the hospital. There is direct correlation between the SCE levels and the clinical severity of poisoning such as presence of ventilator insufficiency and mortality. There was good correlation between improvement in patients motor power with improved serum cholinesterase levels during the time the patients were successfully weaned. There was a generalized increase in serum cholinesterase levels from 1^{st} day of poisoning to subsequent days but without any fixed trend.

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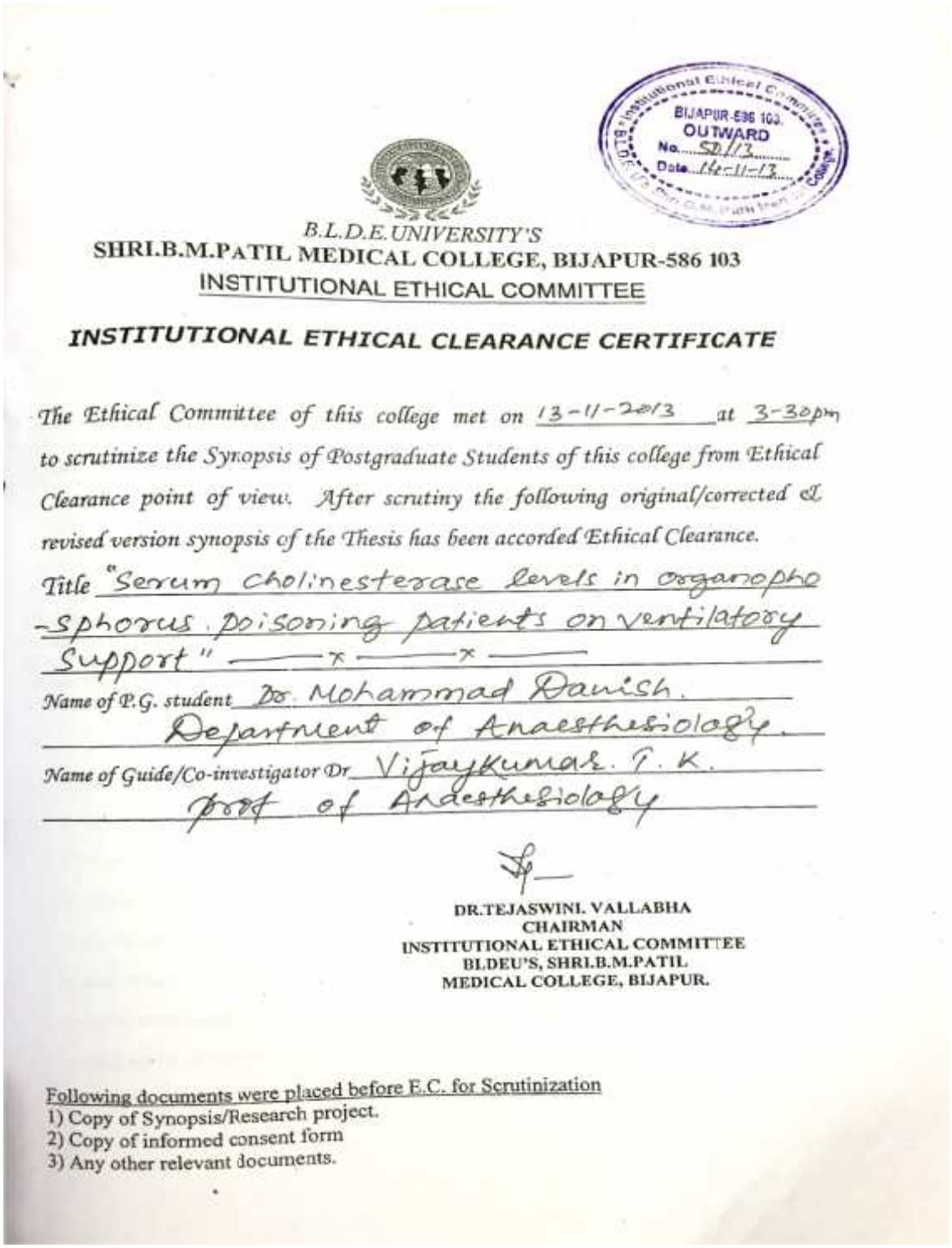
ANNEXURE I

PHOTOGRAPH

PHOTOGRAPH 1; ROCHE COBAS C 311 FULLY AUTO ANALYSER, HITACHI

ANNEXURES-II

ETHICAL CLEARANCE CERTIFICATE



BIJAPUR-586 103.
OUTWARD
No. SD/13
Date 14-11-13

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2013 at 3-30pm to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.

Title "Serum cholinesterase levels in organophosphorus poisoning patients on ventilatory support" —x—x—

Name of P.G. student Dr. Mohammad Danish.
Department of Anaesthesiology.

Name of Guide/Co-investigator Dr Vijaykumar. T. K.
Prof of Anaesthesiology

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURES-III

CONSENT FORM:

I confirm that **Dr Mohammad Danish** has explained to me the purpose of research, the study procedure, that I will undergo and the possible discomforts as well as benefits that I may experience. I have been explained all the above in detail in my own language and I understand the same. Therefore I agree to give consent to participate as a subject in this research project.

_____ _____

(Participant) Date

_____ _____

(witness to signature) Date

ANNEXURES-IV

PROFORMA

NAME: AGE: Yrs. SEX: M/F I.P.NO: POISON:

TIME OF INTAKE : _____AM/PM

TIME OF ADMISSION : _____AM/PM

TIME OF RESP ARREST : _____ AM/PM

TIME OF INTUBATION : _____AM/PM

TIME OF VENTILATION : _____AM/PM

TREATMENT AT CASUALTY: GASTRIC LAVAGE at _____ AM/PM

Inj. ATROPINE: _____ mg. at _____ AM/PM Inj .PAM : _____ mg. at _____ AM/PM

DAY/ TIME	MAP	HR	RR	SpO_2/FiO_2	Crea	Hct	$Na/K/Cl_2$	SCE	UO	PUPIL	SECN	Mot Power	VENT MODE CV/SIMV/CPAP/TPIECE/MASK					Comments
													Vent Mode	TV /rate	PS ab	peep	Mand /BPM	

ANNEXURE V

KEY TO MASTER CHART:

A	-	ALIVE
D	-	DEATH
F	-	FEMALE
M	-	MALE
S	-	Severe
m	-	Mild
M	-	Moderate

Time Interval *: Time Interval between ingestion of OP poison & presentation

Severity*: Based on level of serum cholinesterase

ANNEXURE VI

MASTER CHART

				Time Interval(hr) *	MAP						Heart rate						Serum Pseudocholinesterase value						Severity*	Motor power						Out come
Sl. no	IP NO	Sex	Age		D1	3	5	7	9	11	D1	3	5	7	9	11	D1	3	5	7	9	11		D1	3	5	7	9	11	
1	4421	F	35	7	74	72	65	65	75	82	101	105	106	118	91	92	209	538	1090	2401	2720	3071	S	4	4	4	4	5	5	A
2	5038	F	35	4	81	85	93	89	88		108	90	78	86	76		334	243	722	1861	2214		S	3	3	4	4	5		A
3	32645	M	60	3	71	76	83	78	80		155	118	123	118	110		174	291	668	1511	2366		S	3	4	4	4	5		A
4	34118	M	30	3	86	85	98	106	98		90	102	116	88	96		154	142	136	255	480		S	2	3	3	4	4		A
5	8568	M	27	3	86	83	88	84			116	98	102	124			1621	1709	1965	2566			M	2	3	2	4			A
6	30838	M	79	3	98	83	81	73			130	114	149	94			248	108	123	883			S	4	4	3	3			A
7	16205	M	65	5	97	83	70	84	86		98	96	83	104	96		2118	2301	2555	3408	2727		m	3	4	4	4	5		A
8	4494	F	23	7	58	84	82	80			63	92	102	80			806	700	506	341			S	2	3	3	4			A
9	14176	F	18	3	107	107					101	112					1876	2112					M	3	4					A
10	34494	M	21	4	96	80					118	112					5381	7545					m	4	4					A
11	14148	F	55	6	86	84	50	80			102	110	120	102			860	1258	2011	2345			S	3	3	3	4			A
12	13946	F	25	4	75	80	82	80	92		106	99	104	112	86		563	555	800	620	668		S	1	5					A
13	11256	F	32	4	72	70	82	80			114	93	120	110			354	732	1133	2607			S	3	3	4	5			A
14	7932	M	16	3	82	80	91				69	90	85				487	490	624				S	2	3					A
15	7684	M	28	5	100	80	80	93	95		92	82	80	74	78		966	383	1788	2168	3740		S	3	4	4	5	5		A
16	5524	M	42	4	78	86	80	88	92	86	140	98	98	112	120	110	223	141	155	999	1570	2434	S	3	2	4	3	4	5	A
17	3423	M	35	8	77	87	102	87	94		110	92	154	130	126		3141	4423	4356	4081	4556		m	2	2	2	3	5		A
18	14500	M	30	8	89	87	88	88			126	106	112	102			780	660	650	756			S	3	4	4	4			D
19	4094	F	17	12	90	86	68	83			66	73	85	72			140	779	952	1793			S	1	3	4	4			A
20	5095	M	48	6	88	89					150	140					430	477					S	3	3					A
21	23439	M	19	1	73	78					124	118					2838	1459					m	3	5					A
22	23369	M	32	5	83	73	73				108	131	124				1076	1151	1563				S	2	3	3				A
23	33224	M	60	5	77	67					112	108					1550	1535					M	3	4					D
24	23448	M	62	1	93	71					126	104					380	449					S	3	4					A
25	16576	M	45	12	90	106	91				129	90	82				784	985	2048				S	4	4	5				A
26	7521	M	50	1	67	87					112	90					752	891					S	2	3					A
27	26751	M	21	4	76	84	91	79	71		120	110	132	144	110		373	453	567	1365	2533		S	3	4	3	2	2		A
28	27580	M	22	8	70	40	67				150	140	120				606	524	1344				S	2	5	5				A
29	23129	F	18	3	84	93	86				118	88	87				1607	2409	5328				M	4	2	2				A
30	2722	F	52	1	78	83	80	82	92		114	118	97	108	100		2403	2973	3338	3390	6222		m	3	4	3	4	4		A
31	30882	M	45	8	93	96	98				78	115	112				221	574	1630				S	3	4	4				A
32	15195	F	17	1	82	78	88				124	140	114				2194	4218	5224				m	4	1	3	4			A
33	16300	M	22	12	83	57	52				120	118	122				3286	5864	6429				m	2	3	3				A
34	1294	F	20	7	93	98	73				126	106	114				1906	4418	5207				M	4	3	3				A

35	1603	F	17	9	83	63	72	84			138	122	110	98			580	555	2494	3916			S	2	3					A
36	1674	F	24	3	105	84	77	65			126	157	120	125			327	422	609	856			S	2	2	3	3			A
37	4529	M	20	4	107	107					101	112					565	682					S	3	4					A
38	5214	F	25	1	96	80	93				118	112	92				1375	2281	3780				M	4	4	5				A
39	6998	F	19	7	86	84					102	110					1995	6310					m	3	3					A
40	15651	M	18	3	75	80	88				106	99	110				907	1248	2484				S	1	4	5				A
41	16400	M	45	1	72	70	82				114	93	120				6223	5829	8102				m	3	3	4				A
42	18829	F	27	1	82	80	74				69	90	85				243	272	380				S	2	3	4				A
43	19040	M	60	2	100	80	80	93			92	82	80	74			1926	2323	4790	5382			M	3	4	4	5			A
44	20086	M	62	7	78	86					140	98					1107	3340					M	3	3					A
45	25919	M	40	1	70	88	102				110	92	150				373	521	768				S	2	2	3				D
46	27462	F	25	5	89	87	88	88	86		126	106	112	102	102		169	639	817	1955	2564		S	3	4	4	4	4		A
47	28203	M	25	9	90	86					66	73					5290	6480					m	1	3					A
48	28276	M	22	3	88	89					150	140					3270	3567					m	3	3					A
49	29293	F	25	3	107	107	88				101	112	86				1709	4204	5283				M	3	4	5				A
50	24153	M	26	12	96	80	93				118	112	92				310	352	1083				S	4	4	5				A
51	27342	M	22	5	86	84	50	80			102	110	120	102			1280	2566	4093	4546			M	3	3	3	4			A
52	27825	M	15	8	75	80	86				106	99	92				313	111	1028				S	1	4	5				A
53	38468	M	45	2	72	70	82	80	82		114	93	120	110	96		157	102	103	2480	3105		S	3	3	4	4	5		D
54	39211	M	28	11	82	80					69	90					8119	9123					m	2	3					A
55	17084	F	16	8	100	80	80	93			92	82	80	74			892	1596	3096	3521			S	3	4	4	5			A
56	4873	F	35	1	78	86					140	98					137	248					S	3	2					D
57	6633	F	26	5	77	87	102				110	92	154				195	94	244				S	2	2	3				A
58	7263	F	30	3	89	87	88				126	106	112				141	318	425				S	3	4	4				A
59	8067	M	55	5	90	86	68				66	73	85				185	467	543				S	1	3	4				A
60	23327	F	19	12	88	89	80	93	73		150	140	132	100	100		584	820	2106	3900	4100		S	3	3	4	4	5		A
61	11492	F	25	4	98	83					130	114					233	435					S	4	4					A
62	11902	F	20	8	97	83					98	96					129	219					S	3	4					A
63	13661	M	15	3	58	84					63	92					8901	9232					m	2	3					A
64	14219	M	42	12	107	119					101	112					468	174					S	3	4					A
65	15754	M	64	3	90	86	68	83			66	73	85	72			846	1345	4165	4386			S	1	3	4	4			A
66	17095	M	18	1	88	89	80	93			150	140	132	100			207	1141	2075	2393			S	3	3	4	4			A
67	27254	M	23	3	73	78	91				124	118	90				6131	5525	4502				m	3	4	4				A
68	21218	F	17	1	83	73					108	131					4145	5203					m	2	3					A
69	26583	M	24	6	77	67	85				112	108	116				581	1783	3685				S	3	3	4				A
70	26815	F	25	3	93	71	68	76	72		126	104	128	116	102		300	150	812	1698	2193		S	3	4	3	4			A