INTRODUCTION

Cholinesterase inhibitors (ChEIs) or anti-cholinesterases are chemicals that inhibit the acetylcholinesterase enzyme from breaking down acetylcholine, thereby increasing both the level and duration of action of the neurotransmitter acetylcholine.[1]

1. Acetylecholine:

Acetylcholine (ACh) is an organic molecule that acts as a neurotransmitter in many organisms, including humans. It is an ester of acetic acid and choline, with chemical formula $CH_3COO(CH_2)_2N^+(CH_3)_3$. Acetylcholine is one of many neurotransmitters in the autonomic nervous system (ANS). It acts on both the peripheral nervous system (PNS) and central nervous system (CNS) and is the only neurotransmitter used in the motor division of the somatic nervous system. Acetylcholine is also the principal neurotransmitter in all autonomic ganglia. In cardiac tissue acetylcholine neurotransmission has an inhibitory effect, which lowers heart rate. However, acetylcholine also behaves as an excitatory neurotransmitter at neuromuscular junctions in skeletal muscle.[2]

ACh is synthesized in the cytoplasm of nerve terminals, and acetyl coenzyme A (acetyl-CoA) is synthesized in mitochondria. The reaction acetyl-CoA + choline is catalyzed by choline acetyltransferase, which is synthesized in the soma and reaches the nerve terminals by axoplasmic transport. Since choline must be taken up from extracellular fluid by way of a carrier, this is the rate-limiting step of ACh synthesis.[3]

Vesicles on presynaptic nerve terminals empty their contents into the synaptic cleft when the cytosolic Ca^{2+} concentration rises in response to incoming action potentials (AP). Epinephrine and norepinephrine can inhibit ACh release by stimulating presynaptic α2-adrenoceptors. In postganglionic parasympathetic fibers, ACh blocks its own release by binding to presynaptic autoreceptors, M-receptors.[3]

ACh binds to postsynaptic cholinergic receptors or cholinoceptors in autonomic ganglia and organs innervated by parasympathetic fibers, as in the heart, smooth muscles (e.g., of the eye, bronchi, ureter, bladder, genitals, blood vessels, esophagus, and gastrointestinal tract), salivary glands, lacrimal glands, and (sympathetically innervated) sweat glands.[4]

Cholinoceptors are nicotinic (N) or muscarinic (M). N-cholinoceptors (nicotinic) can be stimulated by the alkaloid nicotine, whereas M-cholinoceptors (muscarinic) can be stimulated by the alkaloid mushroom poison muscarine **(Figure 1).**[4]

Nerve-specific N_N-cholinoceptors on autonomic ganglia differ from musclespecific N_M-cholinoceptors on motor end plates in that they are formed by different subunits. They are similar in that they are both ionotropic receptors, i.e., they act as cholinoceptors and cation channels at the same time. ACh binding leads to rapid Na^+ and Ca^{2+} influx and in early (rapid) excitatory postsynaptic potentials (EPSP), which trigger postsynaptic action potentials (AP) once they rise above threshold.[5]

M-cholinoceptors (M1–M5) indirectly affect synaptic transmission through G-proteins (metabotropic receptors).[6]

M_1-cholinoceptors; occur mainly on autonomic ganglia, CNS, and exocrine gland cells. They activate phospholipase Cβ (PLCβ) via G_q protein in the postganglionic neuron, and inositol tri-phosphate (IP3) and diacylglycerol (DAG) are released as second messengers that stimulate Ca^{2+} influx and a late EPSP. Synaptic signal transmission is modulated by the late EPSP as well as by co-transmitting peptides that trigger peptidergic EPSP or IPSP [6].

M_2-cholinoceptors; occur in the heart and function mainly via a G_i protein. The G_i protein opens specific K^+ channels located mainly in the sinoatrial node, atrioventricular (AV) node, and atrial cells, thereby exerting negative chronotropic and dromotropic effects on the heart. The G_i protein also inhibits adenylate cyclase, thereby reducing Ca^{2+} influx.[7]

M_3-cholinoceptors; occur mainly in smooth muscles. Similar to M_1-cholinoceptors, M_3-cholinoceptors trigger contractions by stimulating Ca^{2+} influx. However, they can also induce relaxation by activating Ca^{2+}-dependent NO synthase, e.g., in endothelial cells.[8]

Termination of ACh action is achieved by acetylcholinesterase-mediated cleavage of ACh molecules in the synaptic cleft. Approximately 50% of the liberated choline is reabsorbed by presynaptic nerve endings.[8]

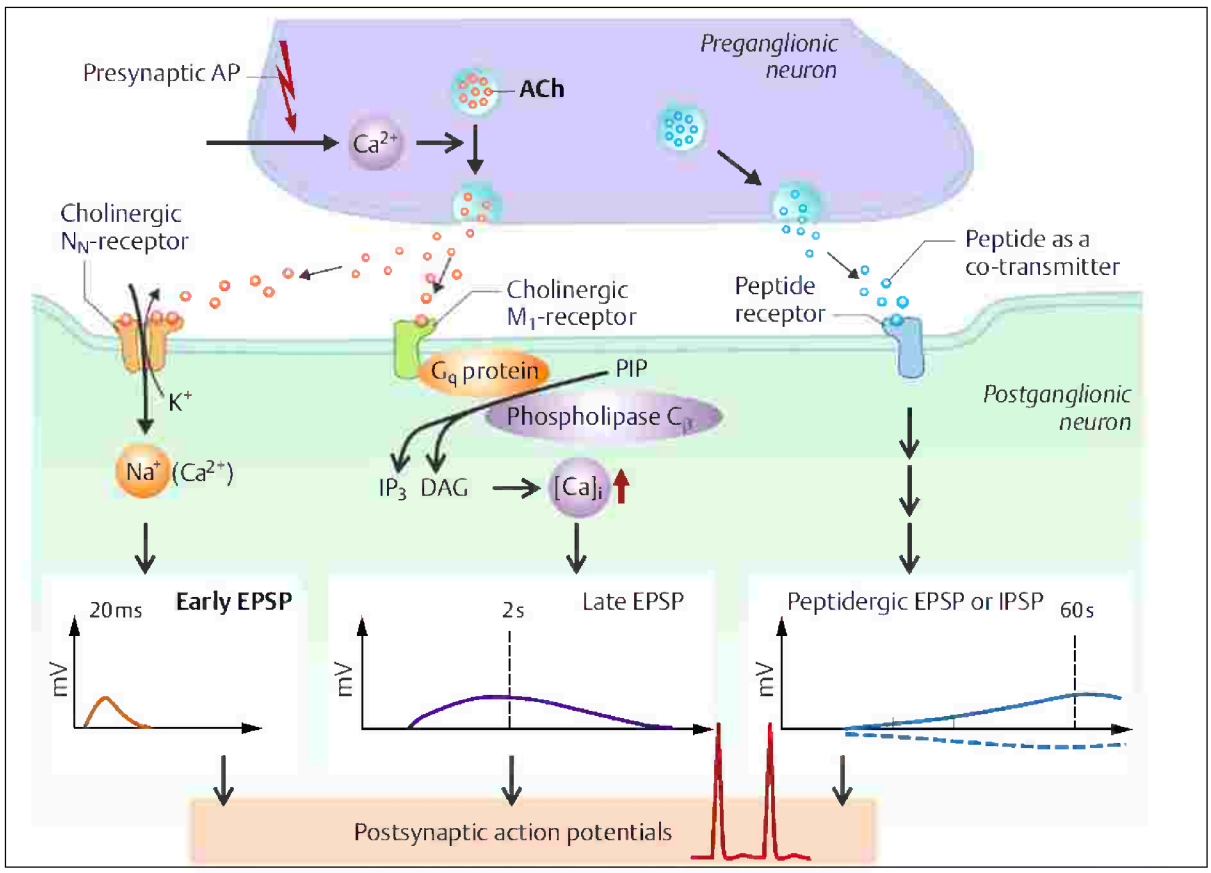

Figure 1: Neurotransmission in autonomic ganglia.[3]

2. Acetylcholinesterase:

Acetylcholinesterase, (AChE), is a hydrolase that hydrolyzes the neurotransmitter acetylcholine. AChE is found at mainly neuromuscular junctions and cholinergic brain synapses, where its activity serves to terminate synaptic transmission. It belongs to carboxylesterase family of enzymes. It is the primary target of inhibition by organophosphorus compounds such as nerve agents and pesticides.[9]

AChE has a very high catalytic activity - each molecule of AChE degrades about 25000 molecules of acetylcholine (ACh) per second. The active site of AChE comprises 2 subsites - the anionic site and the esteratic subsite **(Figure 2).**[10] The anionic subsite; accommodates the positive quaternary amine of acetylcholine as well as other cationic substrates and inhibitors.[11]

The esteratic subsite, where acetylcholine is hydrolyzed to acetate and choline, contains the catalytic triad of three amino acids: serine, histidine and glutamate. The hydrolysis reaction of the carboxyl ester leads to the formation of an acyl-enzyme and free choline. Then, the acyl-enzyme undergoes nucleophilic attack by a water molecule, assisted by the histidine, liberating acetic acid and regenerating the free enzyme.[12]

During neurotransmission, ACh is released from the nerve into the synaptic cleft and binds to ACh receptors on the post-synaptic membrane, relaying the signal from the nerve. AChE, also located on the post-synaptic membrane, terminates the signal transmission by hydrolyzing ACh. The liberated choline is taken up again by the pre-synaptic nerve and ACh is synthetized by combining with acetyl-CoA through the action of choline acetyltransferase.[13]

For a cholinergic neuron to receive another impulse, ACh must be released from the ACh receptor. This occurs only when the concentration of ACh in the synaptic cleft is very low. Inhibition of AChE leads to accumulation of ACh in the synaptic cleft and results in impeded neurotransmission.[14]

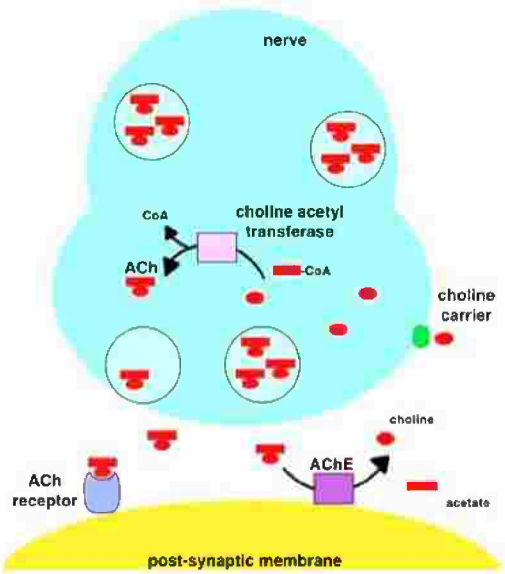

Figure 2: AChe mechanism of action.[9]

3. Acetylcholinesterase inhibitor:

Cholinesterase inhibitors (CEIs) may be irreversible or reversible.

Irreversible inhibitors; these are compounds that are most likely used as chemical weapons (e.g. Sarin and Soman) or pesticides (e.g. OP insecticides). These include:

- Echothiophate, Diisopropyl fluorophosphate, Cadusafos, Chlorpyrifos, Cyclosarin, Dichlorvos, Dimethoate, Metrifonate [irreversible], Sarin, Soman, Tabun, Diazinon, Malathion and Parathion).[15]

Cleavage of OP by AChE leaves a phosphoryl group in the esteratic site, which is slow to be hydrolyzed (on the order of days) and can become covalently bound.[15]

Reversible inhibitors; these are compounds that function as reversible competitive or noncompetitive inhibitors of cholinesterase and occupy the esteratic site for short periods of time (seconds to minutes).[12]

They have most likely therapeutic uses [16]: Tetrahydroaminoacridine (THA) and donepezil are FDA-approved to improve cognitive function in Alzheimer's disease. Rivastigmine is also used to treat Alzheimer's and Lewy body dementia, and pyridostigmine bromide is used to treat myasthenia gravis. [17]

An endogenous inhibitor of AChE in neurons is Mir-132 microRNA, which may limit inflammation in the brain by silencing the expression of this protein and allowing ACh to act in an anti-inflammatory capacity [18]. It has also been shown that the main active ingredient in cannabis, tetrahydrocannibinol, is a competitive inhibitor of acetylcholinesterase [19]. Administration of reversible cholinoesterase inhibitors is contraindicated with those that have urinary retention due to obstruction. [20]

The mechanism of toxicity for cholinesterase inhibitors is essentially the same as the mechanism of action for therapeutic uses. Inhibition of AChE disrupts the dynamic interplay between acetylcholine synthesis, release and degradation. This leads to a net accumulation of synaptic ACh levels with persistent/prolonged activation of cholinergic receptors on postsynaptic cells. This relative increase in cholinergic signaling leads to functional signs and symptoms of cholinergic toxicity. [21]

While all cholinesterase inhibitors are thought to elicit toxicity through this general mechanism, the spectrum of cholinergic signs can be different with different inhibitors, or the same inhibitor with different routes of exposure. Generally, however, one or more of the "classic" signs of cholinergic toxicity will be evident following exposure to any inhibitor that leads to substantial inhibition of tissue AChE activity. The biological responses to cholinesterase inhibitors are predicted upon the distribution and role of cholinergic neurons within the central and peripheral nervous systems. Acetylcholine is the transmitter at pre-ganglionic terminals of both parasympathetic and sympathetic nerves, postganglionic parasympathetic terminals, neuromuscular junction of striated muscle and at various synapses within the central nervous system. Thus, AChE inhibition leads to a relative imbalance of cholinergic transmission at these sites with elicitation of predictable functional alterations [22].

One of the most recognized forms of acute toxicity following exposure to cholinesterase inhibitors is autonomic dysfunction, characterized by excessive secretions at parasympathetic end organs. Excessive stimulation of secretory organs leads to increased secretions at bronchial, lacrimal, salivary, sweat, intestinal and pancreatic sites. Smooth muscles of the ureter also exhibit increased contractions leading to enhanced urination. Together, these responses leading to excessive secretions at multiple sites are a characteristic of cholinergic toxicity. The acronym SLUD or SLUDGE, standing for salivation, lacrimation, urination and defecation, is often used to refer to these types of autonomic signs of toxicity. Loss of regulation at the neuromuscular junction leads to another classic sign of exposure to cholinesterase inhibitors, involuntary movements. The residence of acetylcholine released at the neuromuscular junction is generally limited by the action of AChE such that a single nerve impulse elicits an end-plate potential and subsequent muscle action potential.[23]

With extensive AChE inhibition, acetylcholine molecules can bind multiple nicotinic receptors, prolonging the decay time of the end-plate potential and disrupting the synchrony between end-plate potentials and muscle action potentials. Muscle fibrillations and fasciculations result, and complete muscle paralysis can be noted due to end-plate depolarization.[23]

Ocular effects can also be commonly noted following exposure to cholinesterase inhibitors. Miosis is a classic sign of cholinergic toxicity resulting from constriction of the sphincter pupillae muscle surrounding the iris. In fact, miosis is thought to be the most sensitive indicator of aerosol exposures to some of the most potent cholinesterase inhibitors, e.g., soman.[24]

Constriction of the ciliary muscle, which blocks the accommodation reflex, also increases outflow of aqueous humor and is the mechanism of action for cholinesterase inhibitors for treatment of glaucoma mentioned above. Because of the wide distribution of cholinergic nerves, a host of other signs and symptoms can be associated with cholinesterase inhibitors. Cardiac effects can be prominent and complex. Generally, bradycardia is first observed due to negative chronotropic effects of vagal muscarinic input. However, direct effects on the medullary vasomotor center as well as stimulation of ganglionic nicotinic receptors can influence cardiac function.[25]

Other common signs of intoxication of cholinesterase inhibitors include seizures and convulsions, hypothermia, increased excitability, dyspnea and others. Respiratory failure, through a combination of excessive airway secretions, paralysis of muscles of ventilation, and depression of the respiratory control centers in the pons-medulla is generally considered the primary cause of death following lethal exposures.[25]

4. Organophosphates (OPs):

OPs are one of the most common causes of poisoning worldwide [26].

According to the World Health Organization report, every year 1 million serious accidental and, 2 million suicidal poisonings with insecticides occur worldwide and, of these, approximately 200000 die.[27]

Official data on poisoning in Egypt are difficult to obtain from the scientific literatures. Hospitals concerned with receiving and treating poisoning patients include the public hospitals of the Ministry of Health and population, which are spread all over the country as well as poison control centers in hospitals of some medical colleges in Cairo, Alexandria, El-Menofya, Al-Mansora and El-Menya governorates. [99]

Organophosphates are usually esters, amides or thiol derivatives of phosphoric, phosphonic or phosphinic acids. The general structure is shown in **Figure 3.**

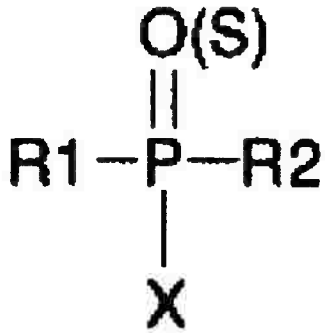

Figure 3. General chemical structure of organophosphate.

Rl and R2 are alkyl-, alkoxy-, alkylthio-, or amido-groups. X is the acyl residue: labile fluorine-, cyano-, substituted- or branched aliphatic, aromatic, or heterocyclic groups. [28]

Effective organophosphates have the following structural features:

- Terminal oxygen connected to phosphorus by a double bond, i.e. a phosphoryl group
- Two lipophilic groups bonded to the phosphorus
- A leaving group bonded to the phosphorus, often a halide

Thiophosphoryl compounds, those bearing the P=S functionality, are much less toxic than related phosphoryl derivatives, which include sarin, VX and tetraethyl pyrophosphate. [29]

OP compounds are absorbed by the skin as well as by the respiratory and gastrointestinal tracts. Dermal absorption tends to be slow but usually lasts for a long time because OPs are difficult to remove from skin. It is more frequent in people than the respiratory route [30]**.** Metabolism occurs through oxidation, hydrolysis by esterases, and conjugation to glutathione. Metabolites may be eliminated via the urine or feces. For example, the pesticide chlorpyrifos is relatively harmless to man because it is rapidly detoxified and excreted. However, a portion of the chlorpyrifos is activated by an oxidative conversion via liver cytochrome P450 microsomal enzymes to form chlorpyrifos oxon, a potent acetylcholinesterase inhibitor. [31]

Organophosphates inhibit AChE, causing OP poisoning by phosphorylating the serine hydroxyl residue on AChE, which inactivates AChE. Irreversible blockage of this enzyme causes acetylcholine accumulation, resulting in muscle overstimulation. This causes disturbances across the cholinergic synapses and can only be reactivated very slowly, if not at

all. Paraoxonase (PON1) is a key enzyme involved in OP pesticides and has been found to be critical in determining an organism's sensitivity to OP exposure. [32]

Paraoxonase (PON1) is a key enzyme in the metabolism of organophosphates. PON1 can inactivate some OPs through hydrolysis. PON1 hydrolyzes the active metabolites in several OP insecticides such as chlorpyrifos oxon, and diazoxon, as well as, nerve agents such as soman, sarin, and VX. PON1 hydrolyzes the metabolites, not the parent compounds of insecticides. The presence of PON1 polymorphisms causes the presence of different enzyme levels and catalytic efficiency of this esterase, which in turn suggests that different individuals may be more susceptible to the toxic effect of OP exposure. The level of PON1 plasma hydrolytic activity provides more protection against OP pesticides. [33]

The catalytic efficiency with which PON1 can degrade toxic OPs determines the degree of protection that PON1 can provide for organism. The higher the concentration of PON1 the better the protection provided. PON1 activity is much lower in neonates, so neonates are more sensitive to OP exposure. [33]

The principal trigger of acute neurotoxicity from exposure to high doses of OP is the inhibition of acetylcholinesterase (AChE). When AChE is inhibited by OPs, it is not able to hydrolyze the neurotransmitter acetylcholine **(Figure 4),** which accumulates in the neural synapse, causing hyper stimulation of cholinergic receptors and resulting in tremors, seizures, respiratory arrest, lacrimation, bradyarrhythmia and even death. OP neurotoxic pathologies result directly or indirectly from organophosphorylation of the active site serine in cholinesterase, causing cholinergic hyperstimulation, and resultant cellular responses.[34]

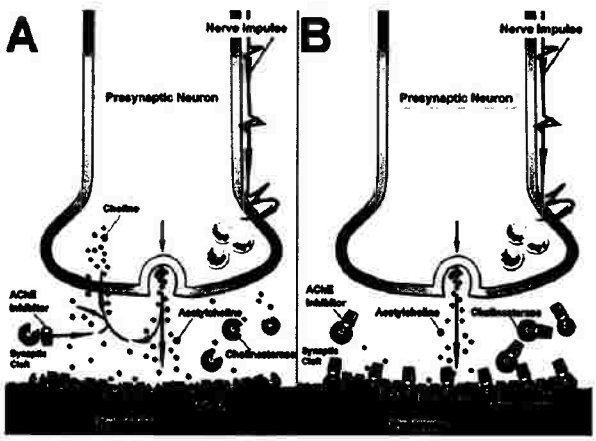

Figure 4. A: The acetylcholinesterase (AChE) enzyme regulates the transmission of nerve signals in the body.

To transmit the nerve signal between neurons, the presynaptic neuron releases acetylcholine, which binds to acetylcholine receptors on the postsynaptic neuron. AChE ends the transmission of nerve signals by breaking down acetylcholine into choline and acetate. **B: OP pesticides "lock" the AChE enzyme.** This prevents AChE from breaking down acetylcholine. Overexposure to OP pesticides inhibits AChE activity, interfering with normal nerve signaling.[34]

Reduced AChE activity is a well-established biomarker of OP exposure. OP inhibition occurs at an essential serine residue synchronous with the ejection of a leaving group to yield a stable covalent bond, forming an OP-AChE adduct. OP-inhibited AChE can reactivate via cleavage of the OP-serine bond or alternatively it can undergo "aging", which a slow, irreversible process is resulting in an OP-AChE adduct that contains a phosphate oxyanion [35].

Patients with OP poisoning may be divided into three groups according to their blood cholinesterase activities and clinical features. "Mildly poisoned" patients have almost normal AChE activity in blood, though their plasma BChE activity may be inhibited up to 50%. "Moderately poisoned" patients retain only 10-50% of normal AChE and BChE activities in blood. "Severely poisoned" patients have nearly undetectable levels of AChE and BChE activities in blood. Symptoms correlate with AChE and BChE inhibition levels, though some clinicians believe that AChE activity measurement is more valuable for diagnosis than BChE activity [36]

The intermediate syndrome:

Acute organophosphate insecticide poisoning can manifest 3 different phases of toxic effects, namely, acute cholinergic crisis, intermediate syndrome (IMS), and delayed neuropathy. Among them, IMS has been considered as a major contributing factor of organophosphate-related morbidity and mortality because of its frequent occurrence and probable consequence of respiratory failure. Despite a high incidence, the pathophysiology that underlies IMS remains unclear. Previously proposed mechanisms of IMS include different susceptibility of various cholinergic receptors, muscle necrosis, prolonged acetylcholinesterase inhibition, inadequate oxime therapy, downregulation or desensitization of postsynaptic acetylcholine receptors, failure of postsynaptic acetylcholine release, and oxidative stress-related myopathy. The clinical manifestations of IMS typically occur within 24 to 96 hours, affecting conscious patients without cholinergic signs, and involve the muscles of respiration, proximal limb muscles, neck flexors, and muscles innervated by motor cranial nerves. With appropriate therapy, that commonly includes artificial respiration, complete recovery develops 5–18 days later. Patients with atypical manifestations of IMS, especially a relapse or a continuum of acute cholinergic crisis, however, were frequently reported in clinical studies of IMS. [37]

The treatment of IMS is mainly supportive. Nevertheless, because IMS generally concurs with severe organophosphate toxicity and persistent inhibition of acetylcholinesterase, early aggressive decontamination, appropriate antidotal therapy, and prompt institution of ventilatory support should be helpful in ameliorating the magnitude and/or the incidence of IMS. Although IMS is well recognized as a disorder of neuromuscular junctions, its exact etiology, incidence, and risk factors are not clearly defined because existing studies are largely small-scale case series and do not employ a consistent and rigorous definition of IMS. [37]

Without a clear understanding of the pathophysiology of IMS, specific therapy is not available. The prognosis of IMS, however, is likely to be favorable if respiratory failure can be promptly recognized and treated accordingly. [37]

A number of measurements exist to assess exposure and early biological effects for organophosphate poisoning. Measurements of OP metabolites in both the blood and urine can be used to determine if a person has been exposed to organophosphates. Specifically in the blood,

metabolites of cholinesterases, such as butyrylcholinesterase (BuChE) activity in plasma, neuropathy target esterase (NTE) in lymphocytes, and of acetylcholinesterase (AChE) activity in red blood cells. Due to both AChE and BuChE being the main targets of organophosphates, their measurement is widely used as an indication of an exposure to an OP. [33]

The main restriction on this type of diagnosis is that depending on the OP the degree to which either AChE or BuChE are inhibited differs; therefore, measure of metabolites in blood and urine do not specify for a certain OP. However, for fast initial screening, determining AChE and BuChE activity in the blood are the most widely used procedures for confirming a diagnosis of OP poisoning. [33]

The health effects associated with organophosphate poisoning are a result of excess acetylcholine (ACh) present at different nerves and receptors in the body because acetyocholinesterase is blocked. Accumulation of ACh at motor nerves causes overstimulation of nicotinic expression at the neuromuscular junction. [33]

When this occurs symptoms such as muscle weakness, fatigue, muscle cramps, fasciculation, and paralysis can be seen. When there is an accumulation of ACh at autonomic ganglia, this causes overstimulation of nicotinic expression in the sympathetic system. Symptoms associated with this are tachycardia, hypertension, and hypoglycemia. Overstimulation of nicotinic acetylcholine receptors in the central nervous system, due to accumulation of ACh, results in anxiety, headache, convulsions, ataxia, depression of respiration and circulation, tremor, general weakness, and potentially coma **(Table I).** [38]

Table (I): Severity grading of OP poisoning based on blood cholinesterase inhibition and clinical features. [36]

Grade	BChE activity (%)	AChE activity (%)	Symptoms	Signs
Mild	40 – 50	50 – 90	Dizziness, anxiety, headache, nausea, weakness, shortness of breath	Failure of accommodation, rhinorrhea, sweating, salivation, coughing, lacrimation
Moderate	10 – 40	10 – 50	(Worsening of the above features plus the following) Restlessness, confusion, dyspnea, disorientation, abdominal pain, diarrhea	Pallor, miosis, lack of concentration, tachycardia, hypertension, muscle twitching, fasciculation, respiratory depression, bronchorrhea, loss of consciousness, bronchospasm
Severe	< 10	< 10	Worsening of the above features	Convulsions, respiratory failure, pulmonary edema, flaccid paralysis Involuntary micturition/defecation Cyanosis, deep coma

When there is expression of muscarinic overstimulation due to excess acetylcholine at muscarinic acetylcholine receptors symptoms of visual disturbances, tightness in chest, wheezing due to bronchoconstriction, increased bronchial secretions, increased salivation, lacrimation, sweating, peristalsis, and urination can occur. [38]

When death occurs, this is believed to be due to respiratory failure due to inhibition of respiratory centers in the brain stem, bronchoconstriction and increased bronchial secretion, and flaccid paralysis of respiratory muscles **(Table II)**. [39]

Table (II): Signs and Symptoms of Acute Poisoning with Anticholinesterase Compounds. [39]

Site and receptor affected	Manifestations
Exocrine glands (M)	Increased salivation, lacrimation, perspiration
Eyes (M)	Miosis, blurred vision
Gastrointestinal tract (M)	Abdominal cramps, vomiting, diarrhea
Respiratory tract (M)	Increased bronchial secretion, bronchoconstriction
Bladder (M)	Urinary frequency, incontinence
Cardiovascular system (M)	Bradycardia, hypotension
Cardiovascular system (N)	Tachycardia, transient hypertension
Skeletal muscles (N)	Muscle fasciculations, twitching, cramps, generalized weakness, flaccid paralysis
Central nervous system (M,N)	Dizziness, lethargy, fatigue, headache, mental confusion, depression of respiratory centers, convulsions, coma

M = muscarinic receptors; N = nicotinic receptors.

The time interval between exposure and onset of symptoms varies with the route and degree of exposure, and the chemical nature of the OP. The first signs to appear are usually muscarinic, which may or may not be in combination with nicotinic signs. While respiratory failure is a hallmark of severe OP poisoning, mild poisoning and/or early stages of an otherwise severe poisoning may display no clear-cut signs and symptoms. Therefore, diagnosis is made through symptom recognition; miosis is observed most often, followed by gastrointestinal symptoms (nausea, vomiting, abdominal pain) and hypersalivation. [39]

The interaction of OPs with AChE has been studied in much detail. OPs with a P=O moiety phosphorylate an hydroxyl group on serine in the active (esteratic) site of the enzyme, thus impeding its action on the physiological substrate **(Figure 5).** The first reaction leads to the formation of a Michaelis complex, while a subsequent reaction leads to phosphorylated AChE. Rates of these two reactions, that are usually very rapid, indicate the affinity of the enzyme for a given OP. The bond between the phosphorus atom and the esteratic site of the enzyme is much more stable than the bond between the carbonyl carbon of acetate (in acetylcholine) at the same enzyme site. [39]

While breaking of the carbon–enzyme bond is complete in a few microseconds, breaking of the phosphorus-enzyme bond can take from a few hours to several days, depending on the chemical structure of the OP. Phosphorylated AChE is hydrolyzed by water at a very slow rate, and the rate of "spontaneous reactivation" depends on the chemical nature of the R substituents. Reactivation decreases in the order demethoxy > diethoxy diisopropoxy. [40]

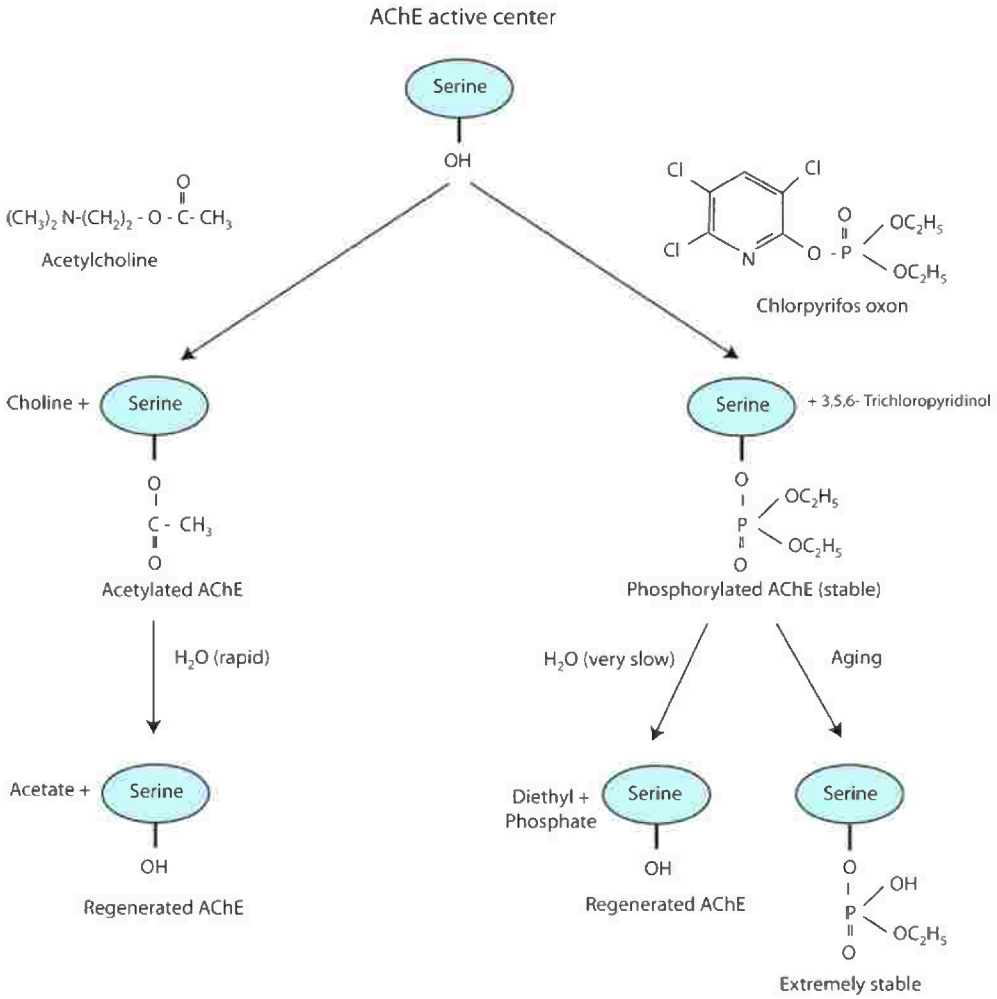

Figure 5: Scheme of hydrolysis of acetylcholine by acetylcholinesterase (AChE) and reaction of chlorpyrifos oxon with AChE.[39]

Whereas water is a weak nucleophilic agent, certain hydroxylamine derivatives, known as oximes, can facilitate dephosphorylation of AChE, and are utilized in the therapy of OP poisoning. Reactivation of phosphorylated AChE does not occur once the enzyme inhibitor complex has "aged". Aging consists of the loss (by nonenzymatic hydrolysis) of one of the two alkyl (R) groups, and the rate of aging depends on the nature of the alkyl group. When phosphorylated AChE has aged, the enzyme can be considered to be irreversibly inhibited, and the only means of replacing its activity is through synthesis of new enzyme, a process that may take days.[39]

Table (III): Drawbacks of cholinesterase activity assays.[39]

Plasma butyrylcholinesterase assays

- Inhibition of butyrylcholinesterase, also called plasma cholinesterase or pseudocholinesterase, does not give information about clinical severity of the poisoning. Many organophosphorus pesticides are more potent inhibitors of butyrylcholinesterase than they are of acetylcholinesterase; butyrylcholinesterase inhibition might occur to a greater extent than acetylcholinesterase inhibition.9 Butyrylcholinesterase assays can be used to detect exposure to an organophosphorus or carbamate pesticide
- Butyrylcholinesterase is produced by the liver, and blood concentrations recover by about 7% of normal each day once the organophosphorus has been eliminated. Daily butyrylcholinesterase assays can be used to monitor when enzyme activity starts to rise again, since this recovery suggests that the organophosphorus has been eliminated
- Variation between commercial assays can make comparisons between studies difficult. The concentration of butyrylthiocholine varies between assays. A high concentration substrate (e.g. 7 mM vs 1 mM) will result in a 30% higher measured activity and a higher background
- Measurement of butyrylthiocholine hydrolysis in the absence of plasma is needed to measure non-enzymatic hydrolysis and hence background values. Not all commercial assays provide such a control. The background amount of spontaneous butyrylthiocholine hydrolysis is affected by its concentration and pH, which both vary between assay kits
- Temperature control is important, because butyrylcholinesterase activity increases by some 4% per 1°C increase in temperature

Red cell acetylcholinesterase assays

- These assays measure acetylcholinesterase expressed on the surface of red cells. Red-cell acetylcholinesterase inhibition is a good marker of such inhibition in synapses and of poisoning severity. This enzyme is measured in whole blood in which butyrylcholinesterase activity has been blocked by an inhibitor. Acetylcholinesterase is present at very low levels in human plasma and serum
- Once red-cell acetylcholinesterase has aged, it only recovers via erythropoeisis. Regeneration at less than 1% per day is therefore much slower than butyrylcholinesterase regeneration. The rate of spontaneous neuronal acetylcholinesterase recovery is unclear, and thus red-cell acetylcholinesterase could be a less useful marker of synaptic acetylcholinesterase activity as recovery occurs
- Reactions between acetylcholinesterase, organophosphorus and oximes will continue if a blood sample is left at room temperature after sampling. The measured acetylcholinesterase activity will then not represent the exact activity in the blood at the time of sampling; leaving samples for different times will give variation in assays. Blood samples must be diluted and cooled immediately after sampling, to stop the reactions. We routinely dilute by a factor of 20 at the bedside by mixing 200 μL of blood freshly drawn into an EDTA tube with 4 mL of cold saline (at 4°C) and then place the sample in a freezer at −20°C within 5 min
- Incubation of an aliquot of blood with a large quantity of oxime (e.g. 100 μmol/L obidoxime) for 15 min before assay will reactivate any acetylcholinesterase that has not aged. Such an assay could potentially be used to establish whether a patient might benefit from continued oxime therapy or from higher doses

- Acetylcholinesterase assays are sensitive to the concentration of oxime and substrate, and pH. Assays with a low substrate concentration, pH 7.4, and therapeutic oxime concentrations will reduce background signal in the assay; however, a blank sample without plasma is needed to quantify the background signal
- Matrix sulfhydryl compounds in red cells (mainly haemoglobin) react with Ellman's reagent. This reaction should be completed by preincubation of red-cell samples with the reagent during temperature equilibration. A higher background activity will be recorded if this procedure is not done
- Monitoring a patient's cholinesterase status after organophosphate poisoning enables the verification of substantial exposure to anticholinesterase agents. In future, such assays could facilitate the decision about when to stop oxime treatment and allow cautious weaning of a patient from a ventilator when butyrylcholinesterase activity is increasing. Studies are underway to confirm the clinical usefulness of this approach.

Management of acute organophosphorus pesticide poisoning:

1. Initial stabilization:

Severe acute organophosphorus pesticide poisoning is a medical emergency. Treatment must ensure that the patient has a patent airway and adequate breathing and circulation. Ideally, oxygen should be provided at the first opportunity. However, little evidence supports the common advice that atropine must not be given until oxygen is available. In hospitals that have no access to oxygen, atropine should be given early to patients with pesticide poisoning to reduce secretions and improve respiratory function. The patient should be placed in the left lateral position, with the neck extended. This position reduces risk of aspiration; helps keep the airway patent, and could decrease pyloric emptying and absorption of poison. Supportive care should include giving fluids and control of blood glucose [41].

2. Gastrointestinal decontamination:

Gastric lavage is often the first intervention poisoned patients receive on presentation to hospital, sometimes at the expense of resuscitation and giving antidote. No evidence shows any form of gastric decontamination to benefit patients poisoned with organophosphorus. Gastric decontamination should only be done after the patient has been stabilized and treated with oxygen, atropine, and an oxime. [39]

Gastric lavage is the most common form of decontamination for organophosphorus poisoning despite the absence of randomized controlled trials to confirm benefit. The rate of absorption of organophosphorus from the human bowel is not known; however, with some pesticides, the rapid onset of poisoning suggests that absorption is rapid, occurring within minutes of ingestion. The time window for effective lavage is therefore probably short. Guidelines for treatment of drug self-poisoning suggest that lavage should be considered only if the patient arrives within 1 hour of ingesting poison.[42]

The relevance of these guidelines to organophosphorus poisoning is unclear but lavage should probably only be considered for patients who present soon after ingestion of a substantial amount of toxic pesticide who are intubated, or conscious and willing to cooperate. Repeated gastric lavages are recommended in some countries to remove

pesticide remaining in the stomach although substantial amounts of organophosphorus are unlikely to remain in the stomach after one lavage.[43]

Ipecacuanha-induced emesis should not be used in organophosphorus pesticide poisoning. Patients poisoned with organophosphorus can rapidly become unconscious, risking aspiration if ipecacuanha has been given. Mechanically induced emesis with large quantities of water risks pushing fluid through the pylorus and into the small bowel, probably increasing the rate of absorption. [44]

3. Principles of therapy:

Treatment includes resuscitation of patients and giving oxygen, a muscarinic antagonist (usually atropine), fluids, and an acetylcholinesterase reactivator (an oxime that reactivates acetylcholinesterase by removal of the phosphate group) **(Table IV).** Respiratory support is given as necessary. Gastric decontamination should be considered only after the patient has been fully resuscitated and stabilized. Patients must be carefully observed after stabilization for changes in atropine needs, worsening respiratory function because of intermediate syndrome, and recurrent cholinergic features occurring with fat-soluble organophosphorus.[42]

4. Efficacy of treatment and outcome:

Although many textbooks regard poisoning with various organophosphorus pesticides to be broadly similar and equally responsive to treatment, differences in chemistry have major consequences for treatment efficacy. The pesticide ingested defines how many patients survive to reach medical attention, how ill they are at admission, effectiveness of oxime therapy, likelihood of recurrent cholinergic crises, or need for respiratory support **(Table IV).** Such variation reaffirms the importance of randomized trials to measure effectiveness of treatments for specific pesticides. [42]

Table (IV): Factors affecting outcome in organophosphorus pesticide self-poisoning.[39]

• Toxicity • Impurities • Formulation • Alkyl sub-groups • Need for activation • Speed of activation and of AChE inhibition • Duration of effect—fat solubility and half-life The above factors have important consequences for the speed of onset of organophosphorus poisoning after ingestion. Ingestion of an oxon organophosphorus that rapidly inhibits acetylcholinesterase will result in early onset of clinical features and respiratory arrest before presentation to hospital, increasing the risk of hypoxic brain damage and aspiration. The conversion of the thioate organophosphorus parathion to paraoxon is so fast that patients can be unconscious in 20 min. Clinical features after poisoning by other thioate organophosphorus, such as dimethoate and fenthion, happen later, giving the patient more time to present to hospital.

5. Muscarinic antagonist drugs:

Although atropine remains the mainstay of therapy worldwide, other muscarinic antagonists have been studied. An important difference between such drugs is their penetration into the CNS. Glycopyrronium bromide and hyoscine methobromide do not enter the CNS, but hyoscine has excellent penetration; atropine enters the CNS, but not to the same degree as hyoscine. [45]

The main adverse-effect of atropine is anticholinergic delirium in patients who receive a high dose. Some physicians therefore prefer glycopyrronium to treat the peripheral effects of organophosphorus without causing confusion. However, its poor CNS penetration suggests that it is ineffective at countering coma and reduced respiration seen in patients with the cholinergic syndrome. [46]

Hyoscine was used successfully to treat a patient with severe extra-pyramidal features but few peripheral signs. Animal studies suggest that it is more effective than atropine for control of seizures induced by inhaled organophosphorus nerve agents. However, extrapyramidal effects and seizures are not common features of organophosphorus poisoning. [42]

Atropine will probably remain the antimuscarinic agent of choice until high-quality randomized trials show another muscarinic antagonist to have a better benefit-to-harm ratio because it is available widely, affordable, and moderately able to penetrate into the CNS. No known randomized controlled trials have compared different regimens of atropine for either loading or continuation therapy. As a result, many different recommendations have been made a 2004 review noted more than 30 dosing regimens, some of which would take many hours to give the full loading dose of atropine. [47]

The aim of early therapy is to reverse cholinergic features and to improve cardiac and respiratory function as quickly as possible. We use a regimen of doubling doses **(Table V),** with the aim of raising the pulse above 80 beats per minute and systolic blood pressure above 80 mm Hg, and rapidly reversing bronchospasm and bronchorrhoea. This regimen allows for as much as 70 mg of atropine to be given in stages to a patient in less than 30 min, resulting in rapid stabilization and low risk of atropine toxicity. [47]

6. Oximes:

Oximes reactivate acetylcholinesterase inhibited by oganophosphorus. Pralidoxime was discovered in the mid-1950s by Wilson and colleagues, and was soon successfully introduced into clinical practice for patients with parathion poisoning. Other oximes, such as obidoxime and trimedoxime, have been developed but pralidoxime remains the most widely used. It has four salts: chloride, iodide, metilsulfate, and mesilate. The chloride and iodide salts are used widely, but metilsulfate and mesilate are used mostly in France, Belgium, and the UK. The chloride salt has advantages over iodide in particular its smaller molecular weight (173 vs 264), which provides 1.5-times more active compound per gram of salt than does iodide. High doses of pralidoxime iodide also puts patients at risk of thyroid toxicity, especially if given for a long period.[48]

Dosage:

Large doses of pralidoxime could have benefit if patients are treated early and have good supportive care. The high-dose regimen was associated with reduced case fatality (1% vs 8%), fewer cases of pneumonia (8% vs 35%), and reduced time on mechanical ventilation (median 5 days vs 10 days). [49]

Pralidoxime may be ineffective in some patients, perhaps because of the specific pesticide ingested, the amount ingested, or the patients' long delay before pralidoxime is given. [50]

Observational studies of pralidoxime and obidoxime suggest that the ability to reverse acetylcholinesterase inhibition with oximes varies with the pesticide ingested. Acetylcholinesterase inhibited by diethyl pesticides, such as parathion and quinalphos, seems to be effectively reactivated by oximes, but acetylcholinesterase inhibited by dimethyl organophosphorus, such as monocrotophos or oxydemeton-methyl, seems to respond poorly. We noted that acetylcholinesterase inhibited by S-alkyl-linked organophosphorus, such as profenofos, is not reactivated by oximes at all **(Figure 6).** [42]

This difference is probably partly because of variation in the speed of acetylcholinesterase ageing **(Table V)** induced by these different pesticides. Interestingly, the Baramati study did not find a difference in benefit of high-dose pralidoxime in moderate dimethyl or diethyl organophosphorus poisoning. Further studies are needed to establish whether this benefit remains for severe poisoning.[49]

Interpretation of clinical evidence regarding oximes should take into account this variability in response of different pesticides. The clinical effects can also be limited by high concentrations of organophosphorus in the blood after ingestion of a large dose the pesticide simply re-inhibits any acetylcholinesterase that the oximes reactivate. Oximes will also not be effective for improvement of outcomes if the patient develops severe complications such as aspiration pneumonia or hypoxic brain injury before treatment. Such complications take place most often with fast-acting pesticides such as parathion and dichlorvos.[51]

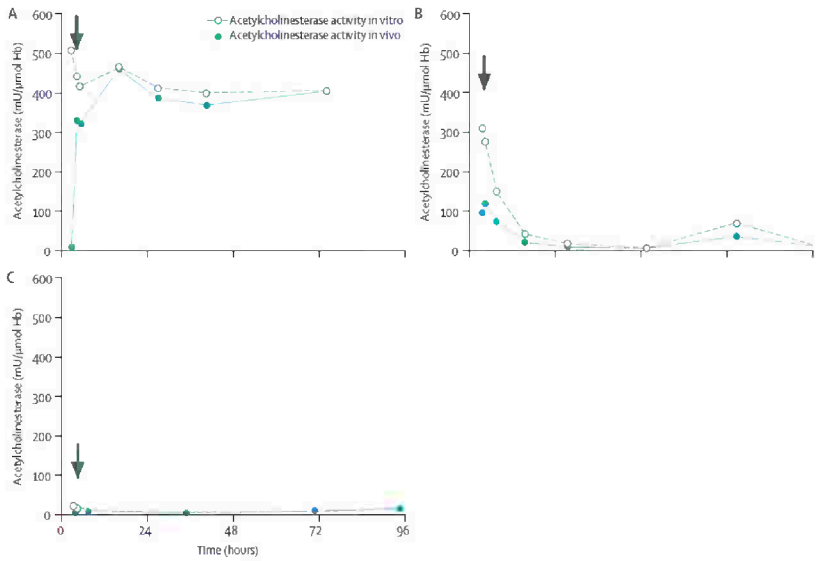

Table (V): Reactions of acetylcholinesterase after inhibition with organophosphorus.[52]

Inhibited acetylcholinesterase reactivates spontaneously but slowly. The half-life of reactivation varies according to the organophosphorus: if dimethyl, the half-life is about 1 h; if diethyl, the half-life is around 30 h. Oximes speed up this reactivation. Unfortunately, if the organophosphorus is present in high concentrations, newly reactivated acetylcholinesterase will be rapidly reinhibited. Whether reactivation or inhibition predominates depends on the type of organophosphorus and relative concentrations and affinities of organophosphorus and oxime. Inhibited acetylcholinesterase can also become aged, by loss of one of the two alkyl groups attached to the bound phosphate. Aged acetylcholinesterase cannot be reactivated by oximes. The half-life of ageing varies according to the inhibiting pesticide: if dimethyl, the half-life is around 3 h; if diethyl, the half-life is around 33 h. Thus ageing has important clinical consequences. If a patient who has ingested a dimethyl pesticide presents to hospital 3 h after ingestion, about 50% of the acetylcholinesterase will already be aged and unresponsive to oximes. A patient arriving after 12 h will have about 94% aged acetylcholinesterase and therefore be unresponsive to oximes. Such a situation is common where patients need to be transferred to a secondary hospital to receive oximes. The situation is better with diethyl pesticides since it takes 33 h for 50% inhibition and oximes can be effective for up to 5 days after ingestion. Ageing seems to take place much more quickly after poisoning with atypical organophosphorus, such as profenofos, that have neither two methyl groups nor two ethyl groups. The half-life of ageing seems to be much less than 1 h, thus oximes are completely ineffective if the patient presents more than an hour or two after ingestion.

Who recommends that oximes be given to all symptomatic patients who need atropine. To ensure a therapeutic concentration, a loading dose of pralidoxime chloride or obidoxime is given, then a continuous infusion. The loading dose of oxime should not be given rapidly as a bolus because this method causes vomiting (risking aspiration), tachycardia, and diastolic hypertension. [53]

7. Benzodiazepines:

Patients poisoned with organophosphorus frequently develop agitated delirium. The cause is complex, with contributions from the pesticide itself, atropine toxicity, hypoxia, alcohol ingested with the poison, and medical complications. Although the mainstay of management is prevention or treatment of underlying causes, some patients need pharmacotherapy. Acutely agitated patients will benefit from treatment with diazepam. [52]

Diazepam is first-line therapy for seizures; however, seizures are uncommon in well-oxygenated patients with pesticide poisoning. Seizures seem to be more common with organophosphorus nerve agents (such as soman and tabun). Animal studies suggest that diazepam reduces neural damage and prevents respiratory failure and death but studies in humans are few. [54]

Creatine phosphokinase (CPK):

1. Introduction:

Creatine kinase (CK), also known as creatine phosphokinase (CPK) or phospho-creatine kinase, is an enzyme expressed by various tissues and cell types. CK catalyses the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP). This CK enzyme reaction is reversible and thus ATP can be generated from PCr and ADP. In tissues and cells that consume ATP rapidly, especially skeletal muscle, but also brain, photoreceptor cells of the retina, hair cells of the inner ear, spermatozoa and smooth muscle, PCr serves as an energy reservoir for the rapid buffering and regeneration of ATP in situ, as well as for intracellular energy transport by the PCr shuttle or circuit. Thus, creatine kinase is an important enzyme in such tissues. Clinically, creatine kinase is assayed in blood tests as a marker of myocardial infarction (heart attack), rhabdomyolysis (severe muscle breakdown), muscular dystrophy, and the autoimmune myositides and in acute renal failure. [55]

2. Serum CK in healthy subjects:

In normal serum, total CK is provided mainly by the skeletal muscle and is almost only of the MM fraction. Total CK levels depend on age, gender, race, muscle mass, physical activity and climatic condition. The 2.5 and 97.5 percentile reference limits have recently been revisited [56]

During fetal life, CK activity is provided mainly by the BB isoenzyme, changing to MM predominance during fetal development. In the newborn, CK serum levels are higher than those in adults and are dependent on gestational age, with values that reach adult levels within the first 10 days of life. In women, CK activity decreases during pregnancy, but increases in late gestation with high values of CK-MB. Young adult males have high serum levels of CK, which decline slightly with age during the geriatric period. [57]

There are marked sex differences in CK serum levels at rest, with lower values in females than in males. After muscular exercise, sex-linked differences are still present, and estrogen may be an important factor in maintaining post-exercise membrane stability, thus limiting CK leakage from the damaged muscle. [58]

Black men usually have higher values than Caucasians, and, although black men usually have a higher body weight and a denser lean body mass, this does not correlate with CK levels; but some studies do not report any differences in the CK serum values between black and white athletes. Anyway, CK activity is related to body mass and physical activity, with resting levels higher in athletes than in sedentary subjects, given the regular training that athletes undergo.[59]

Cold weather induces higher serum CK increases following a standard exercise bout when compared with the same exercise bout at warmer temperatures. [60]

3. CK elevation in pathology:

Monitoring of CK and characterization of its isoenzymes are widely used in the diagnosis of myopathies, cardiomyopathies and encephalopathies. CK, and especially its

MB isoenzyme, is a reliable marker of myocardial necrosis, offering great sensitivity to detect infarct extension to predict worse prognosis.[61]

Patients with neurological conditions such as acute cerebrovascular accidents, proximal spinal muscular atrophy and amyotrophic lateral sclerosis show marked elevation of CK-BB. Elevated CK has also been described in various neuromuscular conditions as a result of muscle damage and necrosis and in many muscular dystrophies such as facioscapulohumeral dystrophy (FSHD) and myotonic dystrophy. [61]

Primary skeletal muscle disorder manifests with pain, fatigue, weakness, and serum CK elevation. The levels of serum CK are different in various myopathies according to the type of disease and the stage of pathology **(Table VI).** Muscular dystrophy shows the highest CK levels. The low CK levels occur in the late stages of the condition because muscle tissue has almost totally undergone fibrotic changes. Other muscular pathologies, such as selenium deficiency or nemaline myopathy, often present only slightly elevated serum enzyme levels. Pain and weakness with mild elevation of enzymes can be due even to the myocardial involvement in other pathology as dilated cardiomyopathy in desmin-related myopathy or polymyositis, which have levels of CK similar to the ones seen in myocardial infarction.[62]

Hypothyroidism is a common cause of endocrine myopathy and should be considered in patients with unexplained persistent elevation of serum muscle enzymes, which are higher in patients with overt hypothyroidism and lower in subclinical hypothyroidism. Many authors suggest to assess thyroid function in patients with muscle weakness or elevation of CK, although clinical signs of hypothyroidism may be absent.[63]

Table (VI): CK values usually found in some muscular pathology.[64]

Muscular pathology	**CK value increases**
Duchenne and Becker dystrophies	25–200-fold
Limb-girdle muscular dystrophy	10–100-fold
FSHD	2–7-fold
Distal myopathy	3-fold
Endocrine myopathy	Up to 10-fold
Congenital myopathies	Slight increase
Metabolic myopathy	Slight increase
Mitochondrial myopathy	Slight increase
Drug-induced myopathies	Slight or no increase

Infective rhabdomyolysis can be another cause of unexplained serum CK increase, most frequently seen in patients with respiratory tract infections and cytomegalovirus infections. Moreover, in pediatric patients, an increase in serum mitochondrial CK can be associated with rotavirus gastroenteritis, probably reflecting the diffusive intestinal epithelial cell damage.[65]

In crush syndrome, CK serum levels have been used as a prognostic tool. In prolonged exposure to cold, as in the victims of avalanche, crush injury to the muscles combines with hypoxia and hypercapnia (secondary to rebreathing and hypothermia) to produce high serum levels of CK. A common cause of exercise-induced rhabdomyolysis is

carnitine palmitoyltransferase deficiency, which impairs mitochondrial oxidation of long-chain fatty acids, often detected by a chance finding of elevated CK levels. The risk of exertional rhabdomyolysis is higher in anabolic androgenic steroids users.[66]

Other causes of serum CK elevation can be intramuscular injections, with the magnitude of serum CK elevation proportional to the injection volume and the drug injected. In compartment syndrome, CK levels are useful to formulate the diagnosis. At surgery, local muscle tissue damage occurs, with CK levels significantly higher in major surgery than in minor procedures. Increased CK levels have been observed following convulsive seizures, heat stroke, administration of statins, which can lead to rhabdomyolysis when used alone, or following interaction with other drugs. Rhabdomyolysis has been observed following the ingestion of herbal medicine.[67]

4. Physiological CK elevation:

Strenuous exercise that damages skeletal muscle cell structure at the level of sarcolemma and Z-disks results in an increase in total CK. When exercise intensity is mild to moderate, the muscle tissue is exercised without marked changes in the membrane permeability: when the exercise intensity exceeds this range, membrane permeability changes and enzymes are released. The boundary of the range of exercise intensity, which the muscle tissue can withstand, is its break point: when loading exceeds a certain limit of muscle ability, CK leaks into the interstitial fluid, is taken up by the lymphatic system and returned into the circulation.[68]

Many factors determine the degree to which serum enzyme activities increase during and after exercise. The highest post-exercise serum enzyme activities are found after very prolonged competitive exercise such as ultra-distance marathon running or triathlon events. Weight-bearing exercises, which include eccentric muscular contractions such as downhill running, induce the greatest increases in serum enzyme activities. There is a breakpoint at 300–500 IU/l of CK serum release after exercise, and the levels of enzyme are associated with distinctive individual musclar properties.[69]

Subjects can be classified into high and low responders. In high responders, the cross-sectional area and volume of the quadriceps femoris muscle were significantly lower than those in low responders. Daily training may result in persistent serum elevation of CK, and resting CK levels are higher in athletes, but the significant increases of CK occurred after exercise are usually lower in trained subjects when compared with untrained subjects. In fact, if athletes and sedentary subjects undertake the same physical exercise test, the CK levels of athletes are lower than those recorded in matched healthy control subjects.[69]

The time of CK release into and clearance from plasma depends on the level of training, type, intensity and duration of exercise. Peak serum CK levels of about 2-fold above baseline occur 8 h after strength training. Increased CK levels after eccentric exercise are associated with muscle injury, with a pronounced increase between 2 and 7 days after exercise.[70]

After prolonged exercise, total serum CK activity is markedly elevated for 24 h after the exercise bout when subjects rest and remains elevated for 48 h when subjects train in the first week post-exercise. The release of CK following eccentric exercise peaked 96 h after the exercise bout, and an additional bout of exercise produces only small increases,

probably from accelerated enzymatic clearance. More intense activity, such as twice-daily football training, leads to significant increase of CK during the fourth day of training. CK levels decrease between days 4 and 10, probably an adaptation to training. A bout of exercise performed 48 h after an initial bout does not change the time course of the CK leakage.[71]

Normally, only CK-MM is present in the serum, but prolonged and strenuous exercise increases the serum activity of all three CK-isoenzymes in the absence of myocardial damage. Probably, the BB-fraction found in boxers is a sign of cerebral damage.[72]

CK serum levels reach their highest values only 5 min after a cycloergometer test, demonstrating that exercise duration rather than fitness levels seems to be related to serum CK, aspartate aminotransferase (AST) and alanin aminotransferase (ALT) activities.[73]

The decrease in the serum enzyme levels depends on the period of rest after exercise, as short-term physical inactivity may reduce both the lymphatic transport of CK and the release of the enzyme from the muscle fibers. Manual lymph drainage after treadmill exercise is associated with faster decrease in the serum levels of muscle enzymes. Another factor that may reduce muscle damage and serum concentrations of CK following prolonged exercise is supplementation with branched-chain amino acids, often used in sports.[74]